to Dr Sahgir Ray
Advisor Health, Planning Comm
with author's best compliments
SCS

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PLAGUE IN INDIA

By
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ABSTRACT

Plague which for centuries ravaged many parts of the world, as also India, is now receding from this country. It prevailed in India in a severe form during the first quarter of this century and then waned gradually to almost vanishing point by the end of the present decade. Nearly the whole of the present-day knowledge on the subject has been acquired during this period, the workers in India contributing to its major share.

Historically the disease is an antiquated one affecting primarily the rodent kingdom and the man getting it from them. In the Indian history the disease is mentioned in *Bhagvata Purana* (1500–600 B.C. ?). It appeared at least thrice in pandemic form, India being severely involved in the last one and Europe in the second with 'Black Deaths' for well-nigh three centuries.

The paper describes the various aspects of infection, carriers, antigenic structure of the organism, prophylaxis, control measures, etc.

INTRODUCTION

Plague which, as one of the most dreaded diseases, haunted the world with its epidemic and pandemic ravages for centuries killing millions of human beings is now on the way to recede from the Indian soil as it did in Europe towards the end of the seventeenth century. In its recent history plague prevailed in India in a serious epidemic form during the first quarter of this century, but it gradually tailed off nearly to a vanishing point towards the end of the present decade (1950–60). Almost the entire knowledge about plague as it stands today has been acquired within this period and it is one of the diseases in which the workers in India have probably contributed a major share. The purpose of this communication is to briefly describe the present status of this knowledge and the relationship it bears to the ultimate conquest of this great pestilence as far as its present position in India is concerned.

HISTORICAL SUMMARY

From the evolutionary point of view all diseases at some stage or the other were prevalent among the animal kingdom from which man got them by legacy or contact. Plague is only one of them, which man even now gets from animals, particularly the rodents. Only under special circumstances is the infection transmitted from man to man when the organism aberrates into a specially virulent form causing pneumonic plague and serious havoc

among human beings. But in so doing it undergoes the risk of complete destruction with the cessation of the epidemic. It can only reappear from rodents or other animal reservoirs.

The first human epidemic on record according to Wu Lien-teh was the outbreak among the Philistines in 1320 B.C. It was characterized by the appearance of emerods in their secret parts as described in I Samuel, V and VI, in the Bible. Some workers, however, doubted this interpretation, but it is not so material when it is believed that the plague was in the animal kingdom and still is, and whenever opportunities were propitious, it propagated among the human beings like so many other diseases. Indian scriptures like the *Bhagvata Purana* (1500–600 B.C.?) gives this disease an earlier antiquity by referring to the deaths caused by an epidemic disease preceded by an epizootic among rats. Men were warned to quit their houses when a rat fell from the roof, jumped about as if it was drunk and died. Similarly it is not essential to know whether the origin of plague was in Central Asia or Central Africa, as described by some of the workers. The moot point is wherever mankind gave up the wild living and started to settle as organized families and tribes the disease started affecting them in groups at times following contact with infected animals either in their wild state or during their attempts at domestication.

More reliable accounts of plague are, however, found beginning from 200 B.C. and it appears from the writings of Rufus, Physician at Ephesus about A.D. 100, that the plague was prevalent in Libya, Egypt and Syria during and before his time, probably as far back as 300 B.C. (Wu *et al.* 1936). The more recent happenings are, however, important in the understanding of the secular trend, the extent and intensities of the problem in the immediate past and the chronological order of events which have led to the present situation. Of the three classical pandemics, the first one occurred during the reign of the Emperor Justinian (A.D. 542). It started from Pelusium, a great trading centre in lower Egypt, from where it spread through North Africa to the Roman Empire on the one side and to Syria, Palestine and Constantinople on the other, and thence to other parts of Europe and Asia reaching London in A.D. 662. It lasted for 50–60 years and killed about 100 million people.

The second pandemic started in the fourteenth century (A.D. 1347) from Caffa in Crimea and spread to China and India on the one side and Asia Minor and North Africa on the other. It was imported to Geneva through the army and from there it spread to other parts of Europe reaching England by A.D. 1349. The disease then ravaged Europe for well-nigh three centuries under the horror of what was known as 'Black Death' taking a toll of 25 million human lives. Evidently pneumonic and septicaemic manifestation with bloody sputum, characteristic cyanosis and skin haemorrhages leading

to black or blue spots on arms, thighs and other parts was quite frequent although the bubonic form must have been more preponderant as in rural England (Greenwood 1911). Retrogression started in the seventeenth century from West to East completely leaving the European stronghold by 1841.

This prolonged pandemic in Europe ushered in new changes in the idea to causation of diseases, their spread and treatment, by revolting against the old system and bringing in the new, what is known as Renaissance in historical term. Quarantine laws were first passed in A.D. 1374 by Count Bernardo of Reggio and by the Venetians in 1403, followed by land cordons and sanitary improvements in the towns.

Millions died in Asia as well, during this pandemic, and India, too, experienced several epidemics. Starting from the north-western part of the country it spread to the central and southern parts and declined towards the close of the seventeenth century leaving behind endemic foci in the foothills of the Himalayas in the district of Garhwal and Kumaon where the disease was locally known as 'Mahamari' and persisted for a long time.

The third pandemic (A.D. 1894) was traced to the reappearance of plague in South China at Yun-nanfu in 1866 from where it reached Canton and Hong Kong by 1894. It then spread far and wide through the marine transport and involved almost all countries except the main lands of Europe by the year 1900. It reached Calcutta in 1895 and Bombay in 1896 and spread from there to almost all parts of India except Orissa, Assam and Eastern Bengal. It prevailed almost unabated till 1918, with a total of 10·25 million deaths. The peak year was in 1907 with 1,315,892 deaths (sp. death rate 5·16 per 1,000). The great epidemic of pneumonic plague in Manchuria started in 1910 and by 1919 all plague epidemics began to decline everywhere except in Java and East Indies. A noteworthy feature of this period of decline was the persistence of plague in some endemic foci, particularly in Asia and India and in some cases in wild rodents as in South Africa, California, Iranian Kurdistan, South America, etc. The spread of infection was rather rapid in this pandemic compared to the slow extension in the second, due to the improved transport and communication facilities.

Chronological History of Epidemics of Plague in India

1500–600 B.C.	Record in *Bhagvata Purana.*
A.D. 1031-32	Plague reached India from Central Asia following invasion of Sultan Mahmoud (Arabian chronicles).
A.D. 1325	Plague in Malabar following invasion of Mahmoud Toghulak and again after Timur.
A.D. 1403	Sultan Ahmed's army was destroyed by plague epidemic in Malwa.

A.D. 1617	Plague reported during the Moghul Emperor Jehangir's reign from the Punjab, Ahmedabad, Surat and Deccan and some other parts of India—described by Edward Ferry, Ambassador to the Moghul Court.
A.D. 1707	Plague in Berhampur.
A.D. 1812–21	In Kathiawar, Gujerat and Cutch—supposed to have been imported from Persia.
A.D. 1836–38	In Merwar and Rajputana—which is known as *Pali* plague.
19th century	Endemic foci in the north near Rawalpindi, in Kumaon and Garhwal (U.P.).
A.D. 1895	In Calcutta—diagnosed bacteriologically on the 17th April, 1898, by Dr. Neild Cook, imported from Hong Kong.
1896	In Bombay, first diagnosed on the 13th October, 1897. From here plague spread rapidly to most parts of India.
1907	Peak year of plague in India with 1,315,892 deaths.
1926-27	Severe epidemic in Hyderabad and Deccan.
1947-48	A temporary rise of incidence in several old foci in India.

Epidemic Behaviour of Plague

The historical review shows that plague allowed to pursue its natural course assumed pandemic form periodically and spread in different directions originally from the endemic home of Central Asia or Central Africa and involved distant parts of the globe. It then receded to its original home leaving behind islands of endemic centres in the various parts of the world, particularly in submontaneous areas. Depending upon the means of communication and conditions favourable for its reception, it took a century or more to reach distant parts of the globe. A number of centuries passed before the disease showed signs of regression, as it also took more or less a century for the process to be completed. With faster and more frequent means of communication this behaviour also changed and the spread of infection in the other pandemic became accelerated manifolds. The important point is that the areas last involved were usually the first to recover unless they presented favourable grounds for sylvatic plague. During pandemic prevalence the disease exhibited epidemicity of varying degrees in different areas according to local conditions. Occasionally in the inter-epidemic periods plague was introduced into non-endemic areas but failed to establish itself and fizzled out in a year or two as in Assam recently (Seal and Bose 1957). Thus plague exhibited two types of secular periodicity, viz. (i) long-term periodicity with intervals of centuries and continuing for centuries or part of a century when once started at such intervals, e.g. recurrence of pandemic plague in the sixth, fourteenth and twentieth centuries, and (ii) short-term

periodicity—a tendency to renew at short but irregular intervals. This is apart from concurrent seasonal periodicity.

The phenomenon cannot be explained in terms of fortuitous happenings or ephemeral local conditions. It rather comes as an outcome of the working of the biological laws. It would be wrong to regard squalor, poverty and absence of sanitary safeguards as the determining cause of pandemic because neither are they confined only to plague epochs nor are they peculiar to plague-infected countries. It is insufficient to ascribe it to spread of infection along a trade route that has probably been operative since time immemorial, while it cannot be denied that the direction and rapidity of spread may be affected by the prevailing methods of transport and communication. The belief expressed by some workers that the ousting of the black-rat by the brown was the cause of decline of the second pandemic may perhaps be partially substantiated although the history of plague in Bombay, Calcutta and other places in India shows that these two species can live together in amity and play their part with fine impartiality in determining the occurrence of plague epidemic of great intensity. What we have found is that whenever the proportions of black-rat as also of *X. cheopis* have been reduced or lessened due to better housing and sanitary conditions, the incidence of plague has also come down or greatly reduced. A comparative study of ward 8 (plague affected) and ward 10 (plague unaffected) in Calcutta by the author during 1954–60 also supported this view.

Again, that the emergence of pandemic is due to a gradual rise of the infection quantum to the epidemic flash point has so far remained as a speculation based on probabilities rather than on experimental proof. Similarly it is a surmise that the decline of pandemic and its gradual retreat to its indigenous home or old (primary) foci is dependent upon the rise of communal immunity as a result of which the disease disappears first from those areas where the conditions are least favourable to its persistence and from other parts of the world outside its indigenous home. According to this assumption the essential cause of the long-term periodicity or of pandemic plague is ascribed to the rise and fall of herd immunity of rodents rather than to the recurrent happenings and ephemeral local circumstances. It may be mentioned here that in 1948-49 plague not only reappeared in Calcutta alone but also in many other towns and cities where plague was absent or at least quiescent for a long time, e.g. Bombay, Lucknow, Gaya, Dhanbad, Asansol, etc. Simultaneously a tendency to recrudesce in places like Hyderabad, Mysore, etc., had been noted. Similar phenomenon was noticed during the last influenza pandemic. The point is how these events or phenomena can be explained or interconnected.

In the author's experience a probable explanation is that when an infection reaches almost to a stage of extinction the organism undergoes a mutation

in virulence, or toxicity or both as a biological process for the preservation of species. By this procedure the effect of rise of immunity or resistance against the existing strain in the community is avoided, and the freshly-emerging organism may then have another lease of life through the epidemic or pandemic which it causes. This is what had happened in influenza in 1957. The phenomenon can be compared with that of locusts. But the question is how the inter-epidemic period is actually bridged over in plague. This was, however, the subject of study by the author in the period 1954–57 which will be referred to later.

Endemic Areas

World

India, Burma, Java, Indo-China, China, Madagascar, South, Central and East Africa, Equador, Brazil, Bolivia, Peru, Venezuela, Argentina, Iraq, Iran, Western Arabia, Western United States (mainly wild rodent foci), Hawaii (*see* Map I).

India

Sub-Himalayan foci in the Punjab, Uttar Pradesh and North Bihar, Madhya Pradesh, Hyderabad (now in Bombay), Bombay, Mysore and Madras (Sharif 1951 ; *see* Map II).

The important landmarks in the development of knowledge about plague is given in Appendix I.

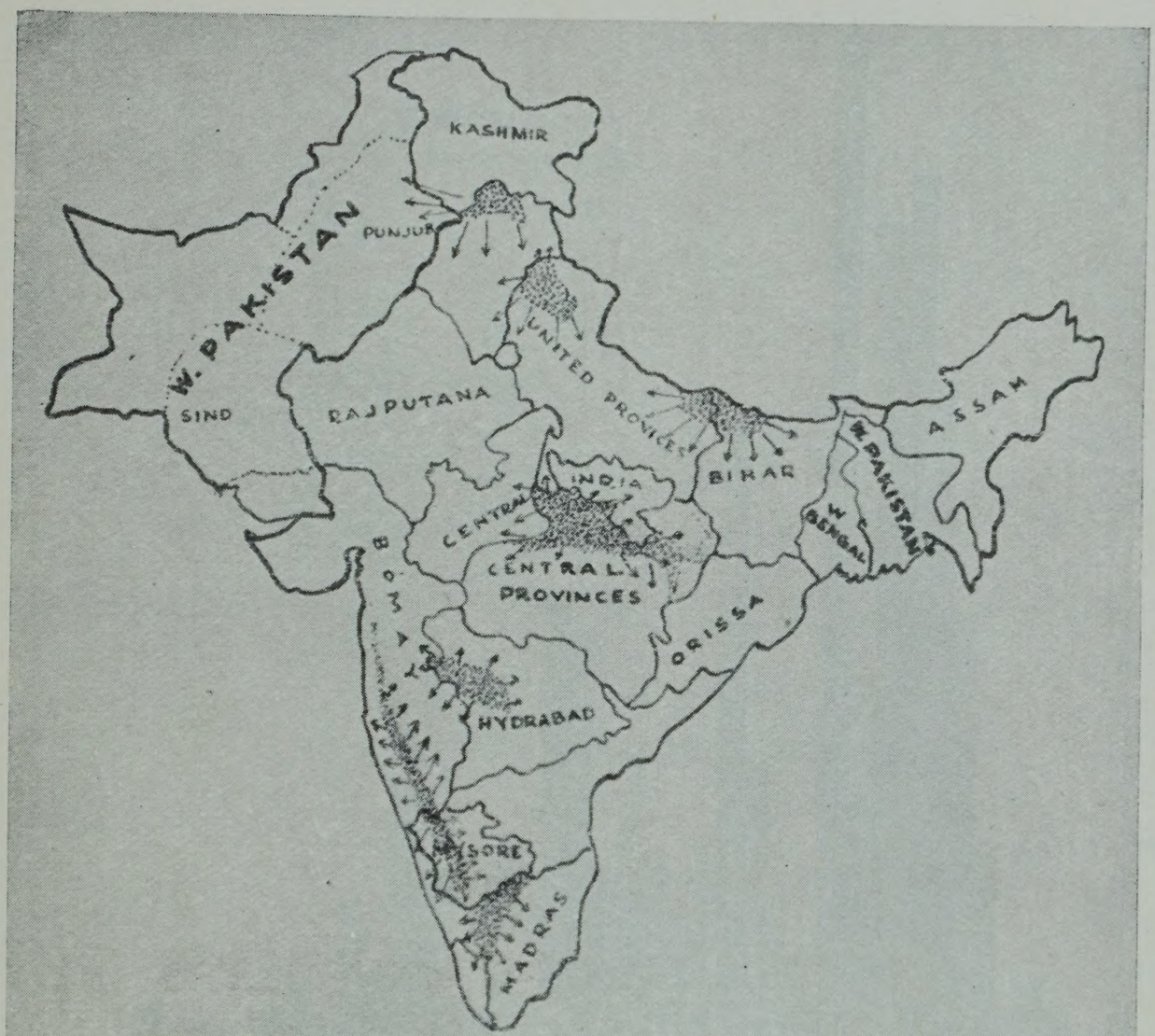
Factors involved in the Epidemiology of Plague

The factors involved in the epidemiology of plague are mainly five, namely the organism, the rat, the flea, the man and the environment, the man being excluded in epizootology. The interaction of these various factors determines the appearance, rise, fall, disappearance and periodicity of epidemic, in other words, its epidemicity and endemicity. Also plague being primarily a disease of the rodents human epidemic is generally always secondary to rat epizootic except the isolated instance of pneumonic plague in Manchuria.

Records of Plague in India since 1895

India got involved very early in the third pandemic in 1895-96 and the peak was reached in about 9-10 years in 1907. Since then the mortality from plague has been one of continuous fall (Graph I) as will be seen from the decennial mortality figures given in Table I and Graph II.

Table I shows that starting from a specific mortality rate of 181·3 per 100,000 during the first decade of plague epidemic in India it came down to 1·8 per 100,000 in the last decade (1949–58). The average annual deaths



MAP II

Plague endemic centres of the Indo-Pakistan subcontinent (Sharif 1951).

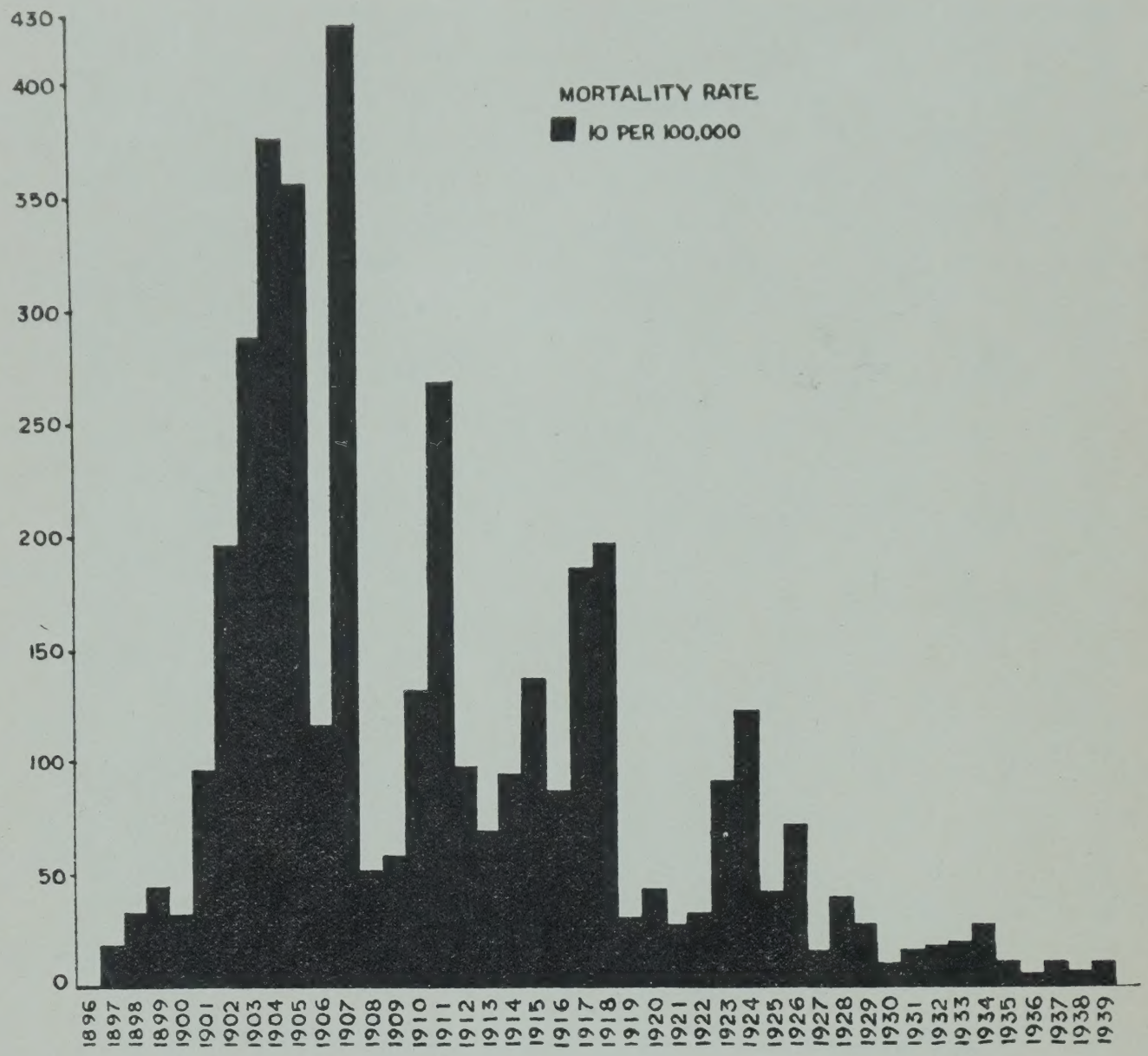
TABLE I

Mortality from plague in India during the period 1898–1957 arranged in decennial periods

Period	Total death from plague	Total population in each period *	Specific mortality rate per 100,000	Plague death as percentage of total deaths, 1898–1957	Average annual percentage of total deaths
1898–1908†	6,032,693	3,291,915,990	183·3	47·47	4·32
1909–1918	4,221,529	3,155,926,382	133·8	33·22	3·32
1919–1928	1,762,718	3,283,195,808	51·9	13·40	1·34
1929–1938	422,880	3,619,458,716	11·7	3·33	0·33
1939–1948	268,596	3,965,924,896	6·8	2·11	0·21
1949–1958	59,059	3,287,649,065	1·8	0·46	0·05
Total	12,767,475	—	—	—	—

* Population was first calculated for each year by intercensus correction and then added together for different periods. Since 1948, population of the areas forming Pakistan was excluded.

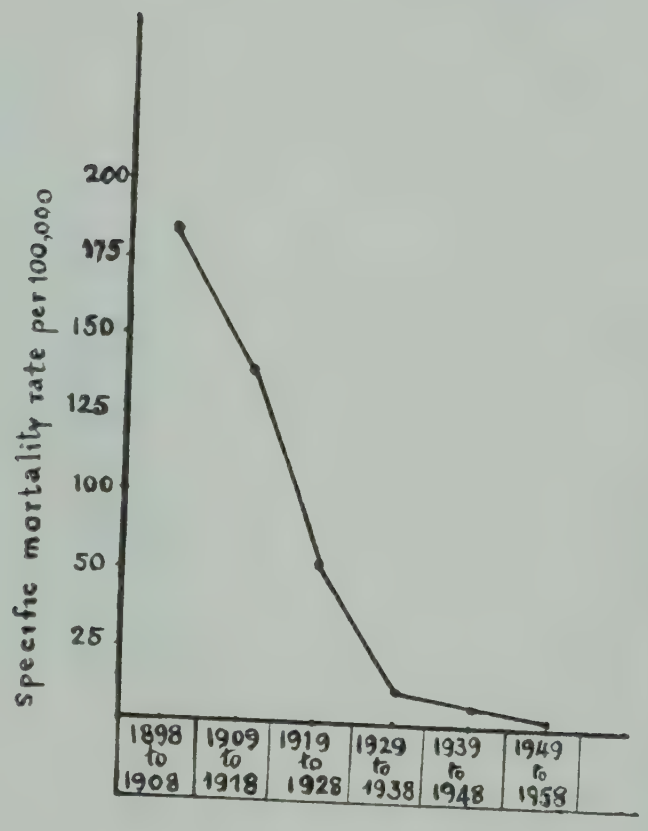
† The first period taken was of 11 years.



GRAPH I

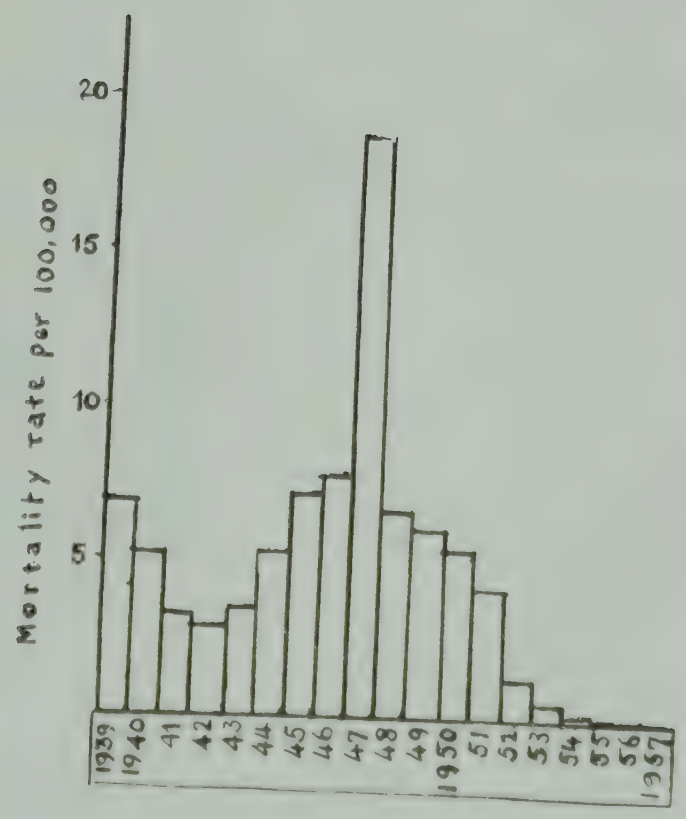
Plague mortality in India per 100,000 population (1896–1939).

due to this disease also came down from 4·32 to 0·05 per cent of total deaths registered. No death from plague has been reported since 1958 except for few stray cases from Mysore and Madras. It must, however, be mentioned that although there was a continuous decline of incidence since 1939 it fluctuated quite alarmingly between 1945 and 1947 and as high as 78,937 human deaths due to plague occurred in India in 1947 as compared to 10,577 deaths in 1942 (*see* Table II and Graphs III and IV). The chronology of State-wise incidences of plague between 1939 and 1957 representing the years 1939, 1942, 1947, 1950, 1953 and 1957 have been diagrammatically shown in six maps included in Diagram I. Plague is thus receding from India. Whether it is a prologue to the final disappearance as it did in Europe, or it is only a phase in the secular trend or it is due to certain measures taken, is a point for consideration. It is on the correct assessment of the present situation that the nature of the steps to be taken now and in future will depend. It



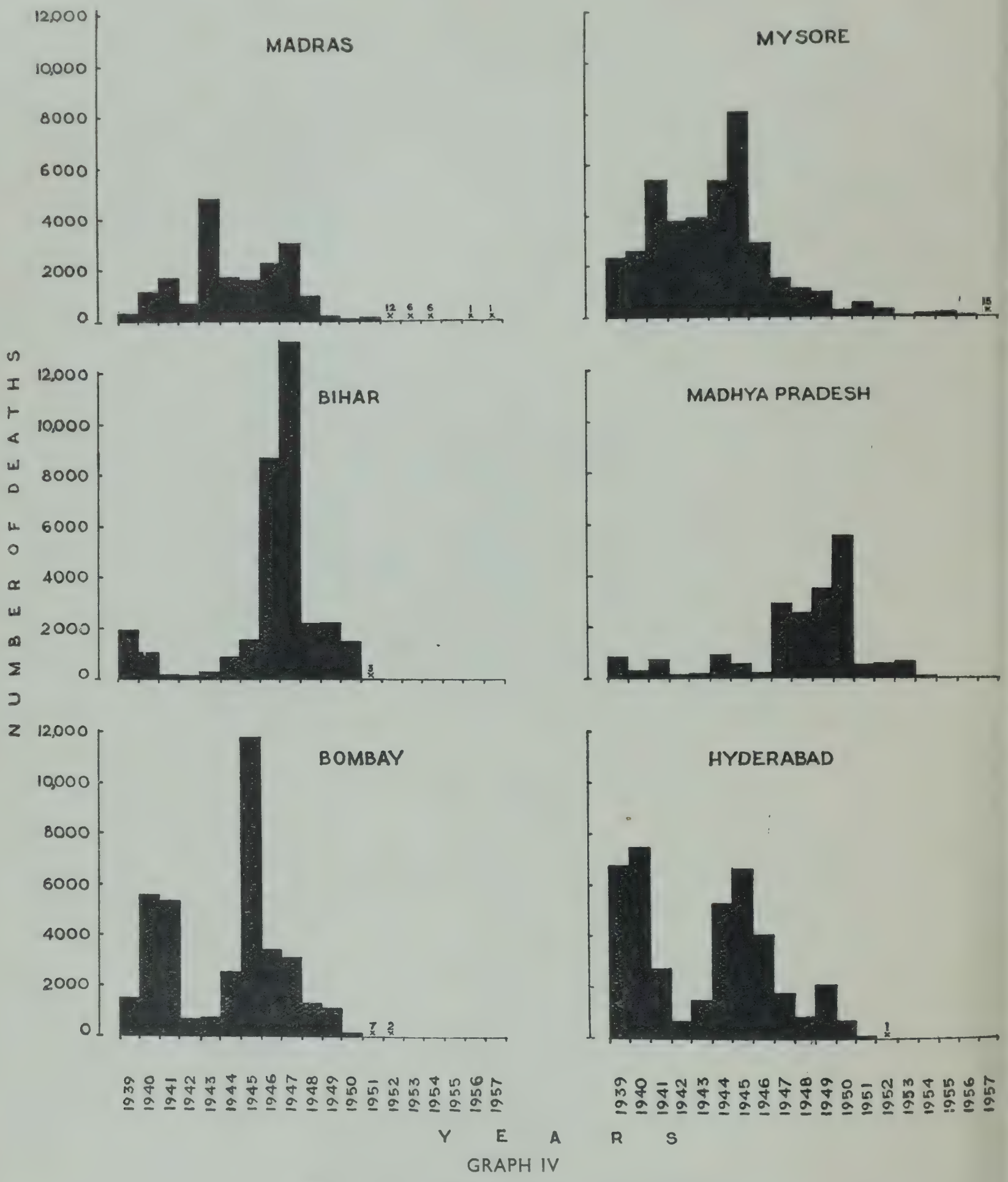
GRAPH II

Decennial death rates of plague per 100,000 population in India (1898–1958).



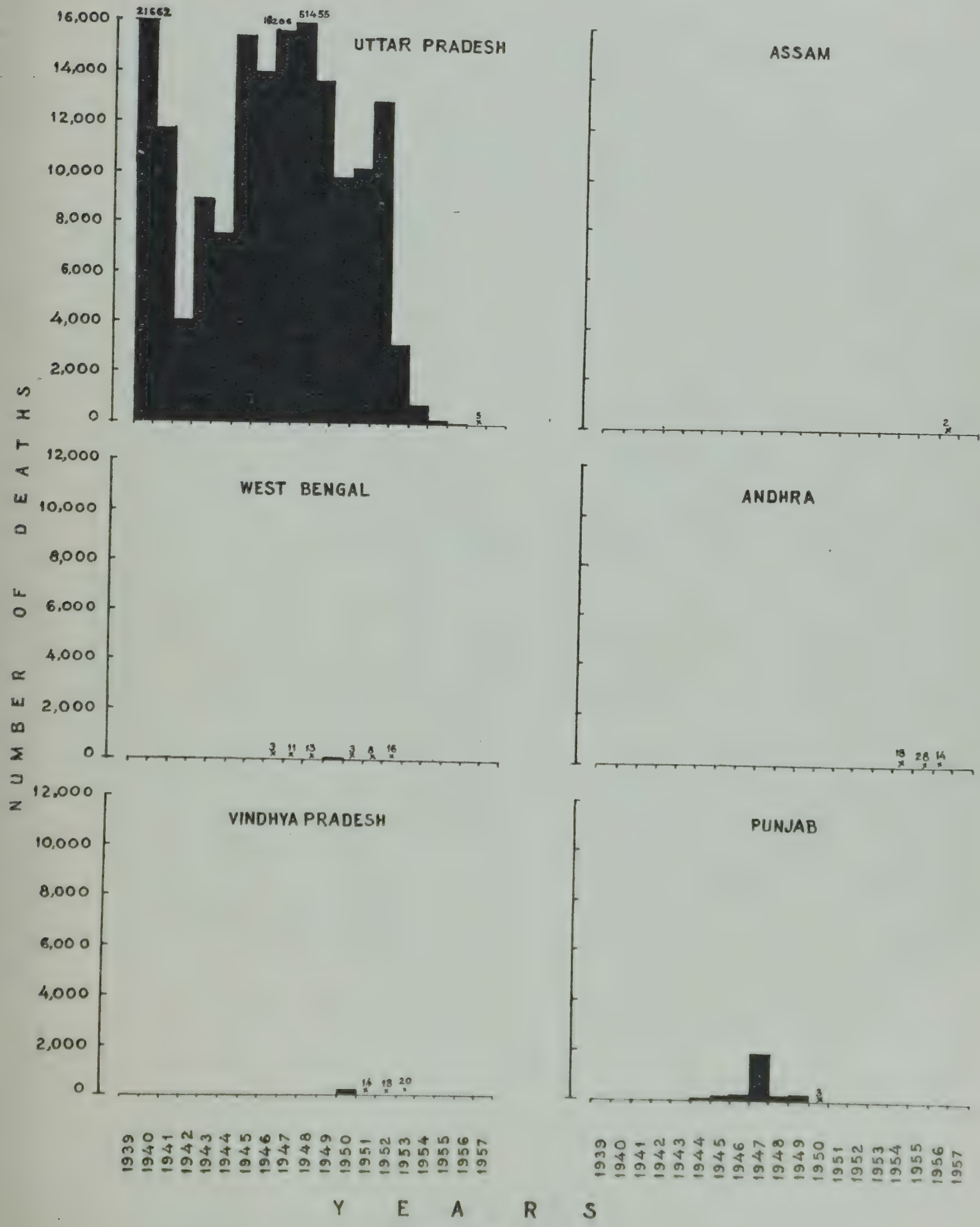
GRAPH III

Plague mortality per 100,000 population in India (1939–57).



GRAPH IV

Plague deaths in different States in India (1939–57).



GRAPH IV—*concld.*

Plague deaths in different States in India (1939–57).

TABLE II

Annual deaths from plague in different States in India during 1939–57

Years	Andhra	Assam	West Bengal	Bihar	Bombay	Hyderabad	Madhya Pradesh	Madras	Mysore	Uttar Pradesh	Vindhya Pradesh	Punjab	Others	Total	Rate per 100,000
1939	—	—	—	1,938	1,472	6,758	852	324	2,352	21,662	—	—	9	26,257	6·88
1940	—	—	—	1,040	5,573	7,500	283	1,169	2,593	11,725	—	—	9	19,799	5·13
1941	—	—	—	129	5,311	2,713	761	1,725	5,417	4,035	—	—	22	11,984	3·08
1942	—	—	—	108	680	657	129	701	3,776	8,953	—	—	6	10,577	2·67
1943	—	—	—	266	715	1,498	144	4,885	3,886	7,556	—	—	11	13,578	3·38
1944	—	—	—	834	2,514	5,263	910	1,738	5,357	15,454	—	61	14	21,526	5·29
1945	—	—	—	1,523	11,779	6,631	575	1,644	8,016	14,024	—	203	3	29,751	7·21
1946	—	—	3	8,689	3,405	4,026	189	2,254	2,894	18,206	—	245	6	32,997	7·84
1947	—	—	11	13,204	3,081	1,791	2,902	3,078	1,502	51,455	—	1,905	8	78,937	18·61
1948	—	—	10	2,142	1,305	811	2,560	978	1,128	13,722	—	211	16	23,191	7·02
1949	—	—	57	2,155	1,139	2,103	3,479	151	982	9,875	—	241	19	20,197	5·76
1950	—	—	3	1,449	146	719	5,568	42	255	10,231	196	3	201	18,813	5·33
1951	—	—	8	3	7	98	513	60	542	12,959	14	—	12	14,178	3·97
1952	—	—	16	0	2	1	575	12	272	3,107	18	—	20	3,905	1·08
1953	—	—	0	0	0	0	679	6	56	762	20	—	—	1,385	0·378
1954	18	—	0	0	0	0	54	6	115	157	—	—	—	296	0·08
1955	28	—	0	0	0	0	0	0	137	29	—	—	—	194	0·052
1956	14	2	0	0	0	0	0	1	52	5	—	—	—	74	0·019
1957	0	0	0	0	0	0	0	1	15	1	—	—	—	17	0·0044

Rajasthan, Kashmir and Jammu—no plague since 1950.
Orissa and other States—no plague.

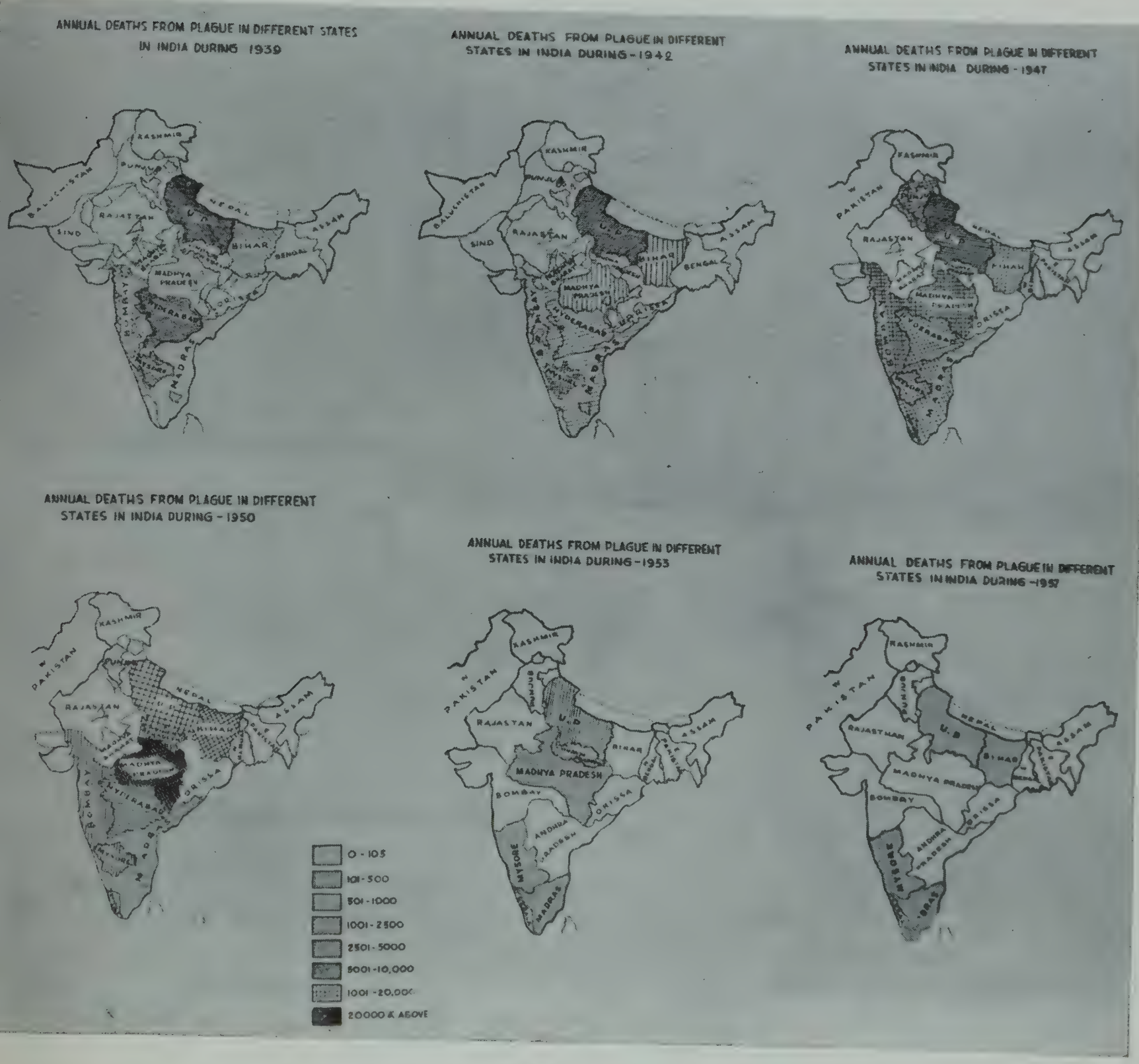


DIAGRAM 1

Annual deaths from plague in different States in India during 1939, 1942, 1947, 1950, 1953 and 1957.

has already been noted that only recently in 1956 a new area in the State of Assam was involved but fortunately aborted.

The portioning of plague deaths on State-wise basis between July 1898 and June 1932 is given in Table III.

It will be seen from Table III that the main brunt of the onslaught fell upon three States mainly, the Punjab, Bombay and U.P., followed by Bihar

TABLE III

The Province-wise distribution of plague mortality between July 1898 and June 1932

Province	Mean population census, 1901, 1921, 1931	Total plague deaths, July 1898–June 1932	Per cent of all India total	Mortality per 1,000 of mean population
Punjab	21,142,793	3,489,123	28·7	165·0
Bombay	19,877,756	2,460,132	20·2	123·8
U.P.	47,164,594	2,911,837	23·9	61·7
Bihar and Orissa ..	34,692,676	1,113,937	9·2	32·1
C.P.	13,991,863	468,165	3·8	33·5
Hyderabad.. ..	12,855,934	425,302	3·5	33·0
Mysore	5,970,446	314,673	2·6	52·7
Rajputana	10,330,957	282,312	2·3	27·8
Madras	42,168,483	227,184	1·9	5·4
C.I. Agency ..	7,653,893	149,941	1·2	19·6
Burma	5,970,446	149,427	1·2	11·8
Other areas ..	38,477,465	109,597	0·9	2·8
Bengal	46,109,157	68,809	0·6	1·5

and Orissa, C.P., Hyderabad and Mysore (of British period) in serial order. The mortality per 1,000 mean population was highest in the first three States mentioned above. Much appears to depend on the social conditions of the patients and attention and nursing available. For instance, in the Hong Kong epidemic case the fatality among the overcrowded, indifferently-fed and unclean Chinese amounted to 93·6 per cent, it was 77 per cent among the Indians, 60 per cent among the Japanese and only 18·3 per cent among the Europeans. Similarly in the first Calcutta epidemic, according to Crake (1908), the mortality varied between 91·2 and 94·5 per cent among the Hindus and between 94·2 and 96·2 among the Muslims whereas the same amongst the Christians was between 51·3 and 53·3 per cent only. The chance of recovery was better in men than in women. The disease also assumed less severity in the vaccinated than in the unvaccinated.

CLINICAL FORMS OF PLAGUE IN INDIA

Plague is essentially bubonic in India. True septicaemic plague is rare except in case of accidental laboratory infection. Primary pneumonic plague is also rare. Generally, it happens after lung-involvement in a bubonic-septicaemic case leading to plague pneumonia and subsequent contacts of such cases develop primary pneumonic plague. Such outbreaks were reported in India (Seal 1949*a*; Seal and Prasad 1949) but they generally remained confined to one or few families only. In fact, the incidence of pneumonic plague remained below 1 per cent and never exceeded 3 per cent of plague deaths in any year since 1895.

Pneumonic form

Wu Lien-teh (1926) tried to explain how this pneumonic form arose. Recently Meyer and Larson (1959) carried out some interesting experiments on the cross-infection among primates (*M. rhesus* and *M. cynomolgus philippinensis*) intra-tracheally infected with plague infection and succeeded in establishing pneumonic infection in 11 per cent and cervical bubonic-septicaemic infection in 58 per cent of those exposed by contact. Henderson (1959) also made similar experiments with guineapigs. Although small particles of infection caused broncho-pneumonia cross-infection was rare and, if at all, ended in septicaemia and death but not broncho-pneumonia. Sokhey suggested that pneumonic plague may be a double infection probably with influenza virus. The author, however, thinks that there is no necessity for such a postulation as once the pulmonary plague develops the organism becomes transmissible through droplets like any other lung infection and thus starts pneumonic plague, but other lung conditions may predispose to pulmonary involvement in a bubonic-septicaemic case.

Urban and Rural Plague in India

Plague is both urban and rural in India, the predominance being of the latter. It appears that plague has failed to gain a foothold in many of the towns of India, perhaps due to untoward climatic conditions and lack of efficient vector (as in Madras and Assam). Regular heavy annual flood may be responsible for keeping certain States like East Bengal free from plague. Another factor which may play an important part is the types and proportion of rodent distribution. With suitable flea vector, larger proportion of susceptible *R. rattus* will facilitate easier and quicker spread among human beings than with other rodents and vice versa. Development of acquired immunity among commensal rat following prolonged outbreaks may either prevent an outbreak or allow simmering zootic plague. Again the urban plague may persist for several years once it is entrenched there in the presence of large rodent population and suitable vectors. If such towns or cities are of commercial importance having traffic connection with other towns and rural areas they often spread plague infection and create secondary plague distributing centres for further propagation of plague. In one essential aspect the urban plague may differ from rural plague in that the latter may be dependent upon commensal, peridomestic or even wild rodent infection and is usually initiated by importation of infection.

The problem of persistence of plague in rural areas still remained a problem for study in India. Kunhardt (1912) tried to explain it by formulating the hypothesis of 'incomplete' and 'complete' plague. In the former case the outbreaks come to an end in the first year before the entire rodent

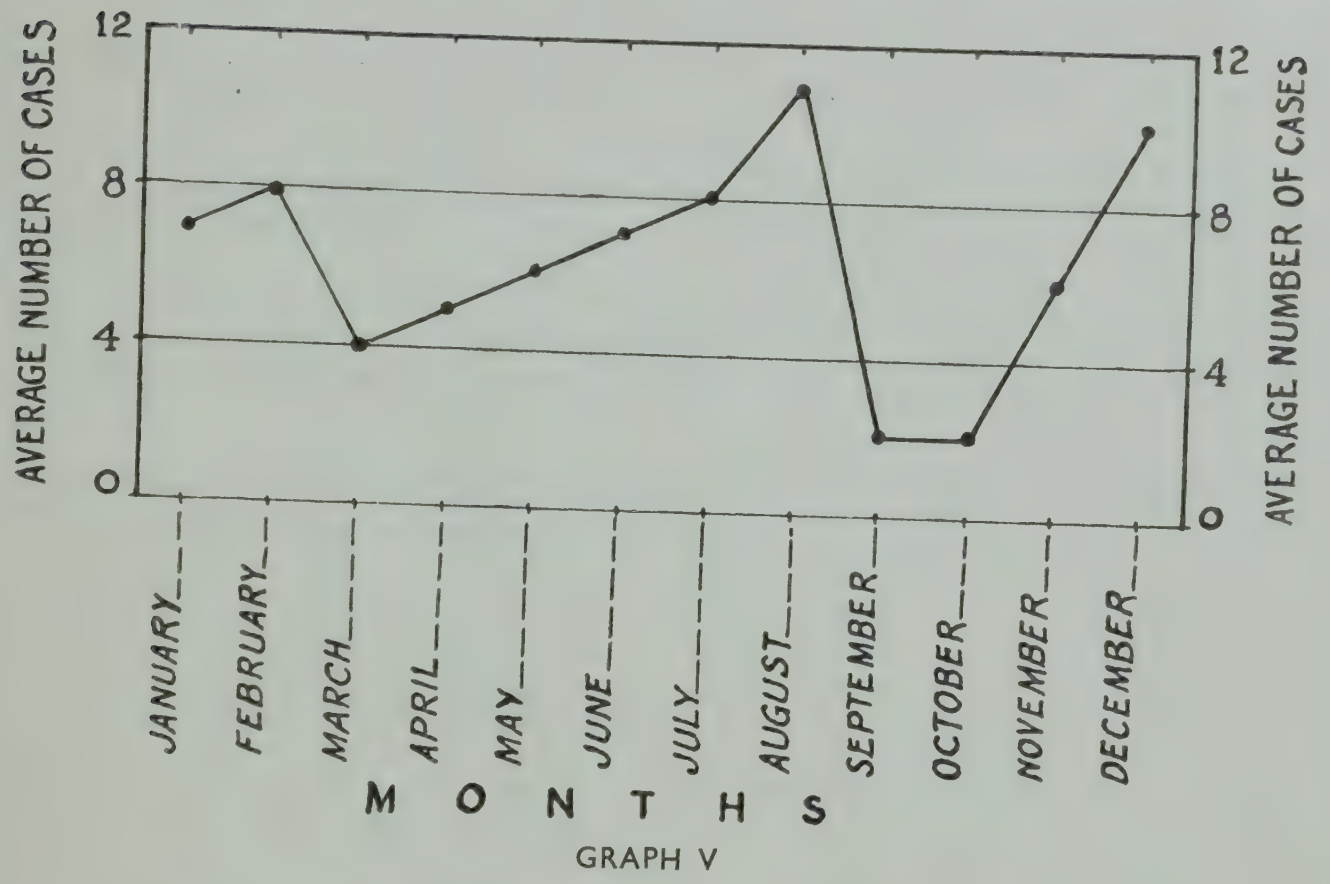
population has been covered and it reappears in the next season and so on till the coverage is complete, resulting in elimination of the susceptibles and development of immunity among the survivors. Actually there were villages in the Cumbum valley (Madras) where plague used to appear year after year. However, Baltazard *et al.* (1958) in their recent observation in U.P. concluded that plague was not endemic there, but there was continuous shifting of the infection from one place to another through contiguity or colony infection among wild rodents, eventually infecting the commensal rats of a village on their path and causing epizootics and human cases. While this observation needs to be confirmed the author believes that multiple factors are involved and the same factor may not be operative in all places equally and the environment, climate and season may also play their respective roles. Besides, all epizootics are not followed by human cases.

Plague may also spread from the affected rural areas to towns through grain traffic or communications as observed by Pollitzer in China (Pollitzer 1954). Again plague may shift from one village to another in the succeeding year and come back to the first village at two or three years' interval (area-wide endemicity ?). According to Sharif (1951) the slow type of epidemic, killing fewer rats, persists longer than the severe epidemic causing heavy rat mortality.

Seasonal Incidences

In bubonic plague the optimum conditions of temperature and humidity as observed under Indian conditions are roughly represented by a mean temperature of 68°–77° F. in association with a relative humidity of the order of 60–70 per cent (some local differences according to different geographical situations may be noticed). There is marked decrease in the incidence of plague with mean temperature rising above 90° F. The largest number of cases occur in the year of highest relative humidity. The seasonal incidence of pneumonic type in Calcutta between 1904 and 1907 is shown in Graph V. The influence of the season is on the numerical prevalence and longevity of rat fleas and on the multiplication of plague organism during the intra-corporeal phase either in rats or in fleas. Rats rarely develop septicaemia below 10° C. (50° F.).

It is thus held that the climate factors, by reason of their influence upon the transmission of infection, are capable of determining the season of the year in which the epidemic of this disease would most likely occur. For instance, at higher latitude the atmospheric temperature attains the critical level only during the late summer and early autumn (as in Kashmir) or at precisely the season of the year when plague epidemic is liable to occur at that latitude. A decrease of latitude is generally associated with earlier occurrence (as in Madras). In the sub-tropical region, on the other hand, where either the temperature or the humidity factor is unfavourable during



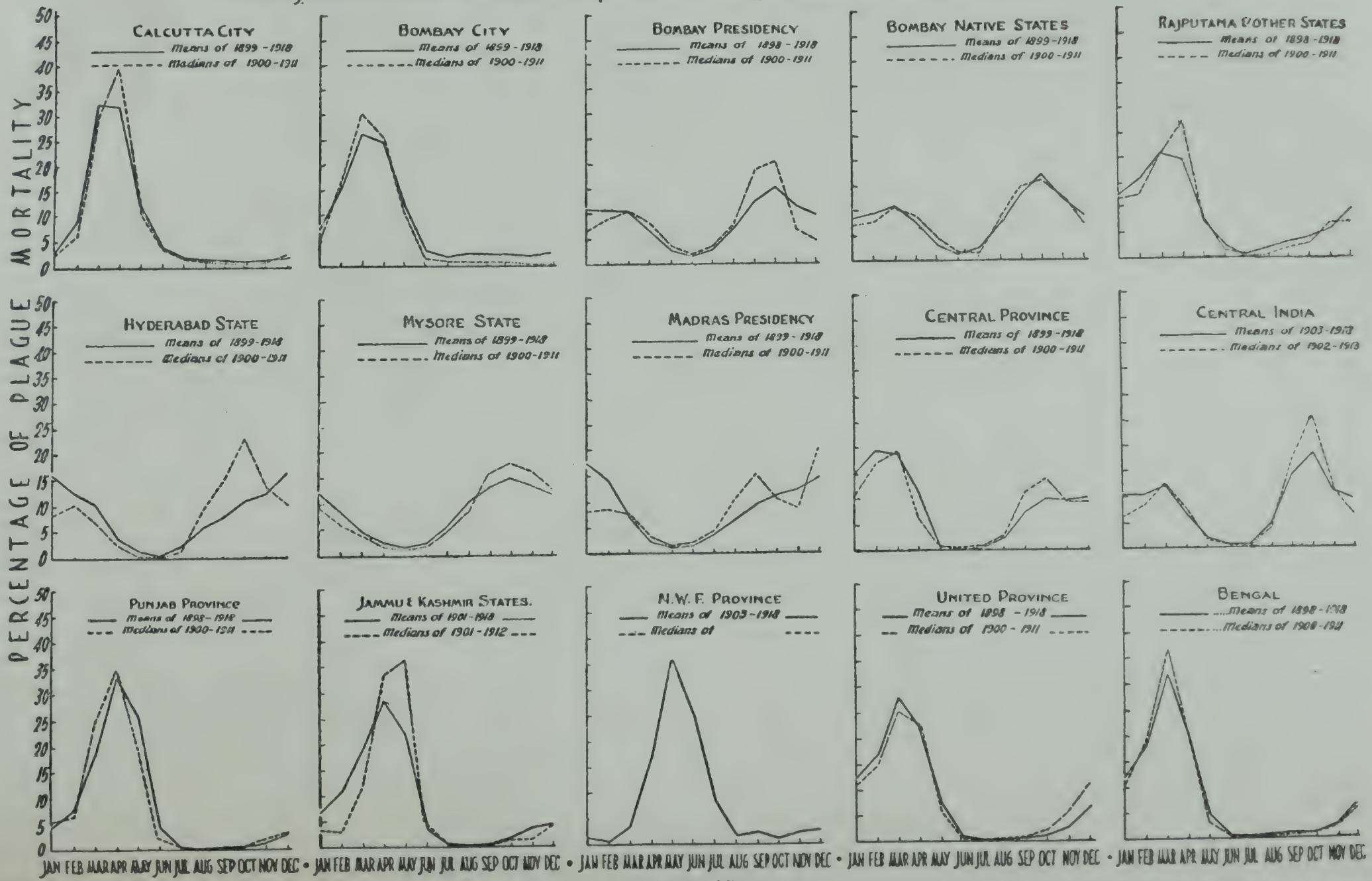
Pneumonic plague in Calcutta (1904–7).

the summer, the plague epidemics have a vernal periodicity (as in northern India).

An analysis of the first 20 years' records of plague epidemics by the author (Seal 1949*b*) in different parts of India since 1896 (Graph VI) suggests two principal seasons of epidemic intensity in a year, viz.—(1) A broad-based single wave, starting in autumn or late autumn and rising to a peak in March or April. In this category is included generally the northern Indian provinces with the Punjab in the north-west and Bengal in the north-east. The city of Bombay (but not the State) has also similar characteristic. (2) Double waves. In this category four characters could be discerned, viz. (*a*) main autumnal wave with a secondary rise in the early months of the year —as in Hyderabad and Mysore States; (*b*) main spring or late spring wave with secondary rise in the autumn, e.g. Kashmir and N.W.F.P.; (*c*) almost equal waves in the autumn and spring, e.g. Bombay province and states. C.P. and Central India and (*d*) the main peak occurring in the early months of the year with occasional secondary rise in the autumn, e.g. Madras province. In brief, the peak of the spring wave is delayed more and more as we move from the Bombay area towards north, north-east or to any higher altitude like Kashmir and also there is a tendency to replace the double waves with a single one. On the other hand, the peak comes earlier as we move towards the south or south-east. For instance, in some outbreaks in N.W.F.P. the peaks occurred in May and even in June whereas it was in

Seasonal Distribution of Plague Mortality in Different Cities & Provinces in India

(Monthly means and medians expressed as percentage of yearly totals)



GRAPH VI

January or February in Madras province. The two months which show the least incidence in India are June and July, and also January and February where the winter is severe (*see* Table IV). In Calcutta out of 20 epidemic waves since 1896, 4 reached the peak in March, 12 in April and 4 in May. On the whole, in the northern Indian belt the maximum plague mortality occurred between March and May and the disease tended to die out with the onset of hot weather, i.e. high temperature with reduction of humidity, both of which are inimical to the longevity of rat flea and to its power to transmit infection. The most favourable period is therefore autumn and spring, particularly the latter. This period has been found to be related to the normal growth curve of the *X. cheopis*, the vector fleas (Seal 1955, 1958*a*).

Reservoirs of Infection

The rodents which have been found associated with plague infection are generally classed as (1) wild rodents, (2) commensal or domestic rodents and (3) peridomestic or semi-domestic rodents, according to their ecological behaviour. Only domestic rodents are involved in pandemic plague and in most of the epidemics. The role of wild rodents seems to be mainly in the maintenance of plague on long-term basis with occasional transfer of infection through semi-domestic rodents to the domestic rodents and thus to perpetuate epizootics among the commensal rodents and eventually among human beings.

Wild rodents

Since 1929 the list of rodents other than that of rats and mice, known to suffer from natural plague and also of those suspected of carrying the infection, has grown consistently and lately the W.H.O. has published a comprehensive list of rodents and lagomorpha containing more than 200 species or subspecies involved in the natural plague infection. During this period foci of wild rodent plague have been defined in Central Asia, South-East Russia, South Africa, East Africa, South America, U.S.A., and recently Iranian Kurdistan. The characteristics of the principally-involved species such as movement range, susceptibility and resistance, role of interaction, effect of density, seasonal incidence of plague and trend of epizootics, modes of spread of infection and the interrelationship between wild rodent and rat plague have been studied.

As far as India is concerned, the earlier workers could not secure any convincing evidence for the independent existence of wild rodent plague in India except occasional positive findings in *Funumbulus pennati* (Simond 1898; Hirst 1922), in *Tatera indica* and *Mus booduga* in the Cumbum valley of Madras by George and Webster (1934) and in *Tatera indica* and *Mellardia meltada* in Bombay by Sharif and Narasimham (1941). On the other hand,

TABLE IV

Number of plague outbreaks in any month in the period 1898–1918 in different parts of India

Province or city	Years of study	Months												Months of lowest incidence	Start of rise	Remarks
		J	F	M	A	M	J	J	A	S	O	N	D			
Bombay City	20	—	—	7	13	—	—	—	—	—	—	—	—	June-July or Nov.-Dec.	Nov.-Dec. or Jan.	During first six years rise was in February; in remaining years generally in January
Bombay Province	21	3	4	11	2	—	—	—	—	2	16	1	—	June-July	July-Aug. and Jan.	Double rise: autumn and spring
Bombay States	20	1	3	11	4	—	—	—	—	3	13	2	—	June-July	July-Aug. and Jan.	Double rise: autumn and spring
Hyderabad States	21	4	6	2	—	—	—	—	—	—	9	3	4	June-July	July-Aug. or Jan.	Irregular double rise: fall of peak often continued until June-July or next year
Mysore States	20	7	2	—	—	—	—	—	3	4	7	4	—	May-June	July and Jan.	Generally continuous fall from autumn peak
Madras Province	20	12	4	—	—	—	—	—	1	4	1	2	1	May–July	Aug.-Sept. or Dec.-Jan.	Double rise in 8 instances with main rise in winter
Central Province	20	1	8	5	—	—	—	—	—	1	7	3	2	June-July	Aug. or Jan.	Double rise in 10 instances: equal number of peaks in autumn and winter
Central India	16	1	5	9	—	—	—	—	—	2	11	2	—	May–July	Aug. or Jan.	Double rise in all years
Rajputana States	21	—	1	10	7	—	—	—	—	1	2	1	—	June-July	Aug.	Double rise in 4 instances only. Main rise in spring
Punjab	21	—	—	3	15	3	—	—	—	—	—	—	—	July-Aug.	Sept.-Oct.	Peak only in spring
Kashmir	18	—	—	2	7	7	—	—	—	—	1	1	1	July-Aug.	Oct. or Nov.	Double rise in three instances only, but peaks very small. Main rise in the late spring
North-West Frontier Province	10	—	—	—	1	5	2	—	—	—	—	1	2	Jan.–March	March or Oct.-Nov.	Double rise once only with very small peak in December
United Provinces	19	—	—	18	1	—	—	—	—	—	—	—	—	June-July	Sept.	Peak only in early spring
Bengal (including Bihar)	20	—	—	13	6	1	—	—	—	—	—	—	—	June-July or Aug,–Dec.	Aug.-Sept. or Jan.-Feb. (later years)	Peak only in spring
Calcutta	20	—	—	4	12	4	—	—	—	—	—	—	—	July–Oct. or Aug.–Dec.	Nov.-Dec. or (later years)	Peak only in spring

Rao (1947) in Hyderabad found *Tatera indica* very susceptible and *R. rattus* resistant. Previously Sokhey and Chitre (1937) came to the same conclusion. Recently, however, Baltazard *et al.* (1958) found infection in *Tatera indica* in certain parts of Uttar Pradesh.

Peridomestic and commensal rats

Infection was, however, more commonly found among the peridomestic rats like *B. bengalensis* (Hossack 1906), *B. indica* (Indian Plague Research Commission 1907 and 1910) or *B. bengalensis* and *B. malabarica* (George and Timothy 1941). The author also during his investigational work on the recent Calcutta outbreak (1948–51) found evidence of dormant infection among the peridomestic rats, *B. bengalensis*. Chowdhury (1957) isolated such infection from the rat. The three important species among the commensal rodents (murinae) which are usually involved in plague epizootis are *R. rattus*, *R. norvegicus* and *Mus musculus* and occasionally *Suncus murinae* (musk rat). Although a fairly intensive study has been made about their ecology, there is enough scope for further study depending upon the area, geographical, climatic, soil and physiographical conditions. Among the important items of ecological studies made may be mentioned: (1) breeding habits, (2) population dynamics, (3) movements, migration and

TABLE V

Types of rats and their percentage distribution

City	Year	Observer	*R. rattus*	*R. norvegicus*	*B. bengalensis*	Bandicota	Others
Calcutta	1906	Hossack ..	14·0	26·0	60	—	—
	1936	Rao	13·5	22·0	27·3	—	37·2
	1948–50	Seal and Bhattacharji ..	13·3	9·0	75·8	1·7	—
	1956	Chowdhury ..	7·3	9·0	79·9	0·3	—
Bombay	1910	Indian Plague Research Commission ..	66·2	28·7	1·0	—	4·1
	1929-30	Webster and Chitre ..	79·1	11·0	9·9	—	—
	1956	Deoras & Gokhale (Haffkine Institute, Bombay)	22·9	15·9	49·2	—	12·0
Madras	1910	Indian Plague Research Commission ..	49·4	—	—	—	50·6
	1931	King and Pandit	98·8	—	—	—	1·2

transportation, (4) relative distribution of different species in a locality, (5) resistance and susceptibility, and (6) maintenance of infection. In regard to the breeding habits the author's observation in Calcutta shows that there is an accumulation of rat population during the first three months of the year, the peak month being April or almost coinciding with the plague epidemic season (Seal 1958*a*). The study of rat movements showed that their movement is mainly intramural and intercolonial but they trace their path back to the colony when released seven or eight miles away. The author also believes that there is metastatic movement also at the initial stage of an epizootis which helps propagation of infection to even distant parts (Seal 1958*b*).

In recent times a changing pattern of distribution of rodents has been noted in Calcutta, Bombay and Madras as will be seen from Table V.

Movements of Rats in Relation to Dissemination of Plague Infection

Under normal conditions the commensal rats in the urban area move within a very narrow orbit (Davis 1951; Bhattacharji and Seal 1954; Kartman and Lonergan 1955). In the rural areas they generally have a strictly limited home range but, as noted by Venables and Leslie (1942) and Davis (1951), they are able to make seasonal migration and, according to Macchiavello (1948), they are apt to undertake progressive migrations covering wide distances. Some workers also have noted mass migration, though not common. In this connection the author made an interesting observation in the city of Calcutta during the last 1948–50 outbreak of plague (Seal and Bhattacharji 1960*a* and *b*). Suspecting the dispersal of rats at this very early stage of the epizootis carrying infection simultaneously to distant parts experiments conducted with 'marked' rodents caught from the field and released at long distances from the places of their habitat showed that the rats so removed had a tendency to go back to their home colonies, even though they had to cover long distances for the purpose. He also observed that though there was considerable intermingling of neighbouring colonies, ordinarily the distance covered did not exceed 50 yards and was never more than 200 yards. In agreement with the observation it was found that the larger percentage (61·4 to 72·0 per cent) of confirmed human plague cases could be explained on the basis of rat deaths detected between 50 and 200 yards and 22 per cent beyond 200 yards; only 10·4 per cent remained unexplained. Therefore wherever plague-affected rats were detected it was necessary to apply intensive control measures within an area of 200 yards.

The problem of passive transference of rats through marine and land traffic, particularly of food grains, cotton, jute, etc., is well known.

Resistance of the Rat Population

As a result of a series of outbreaks of plague in a locality the surviving rat population acquired herd resistance against the disease due to active immunization and possibly also to natural selection and a stage is reached when the major section of the population acquires resistance, and the plague completely ceases there for the time being. This was one of the ways how plague subsided in Bombay. The results of resistance test of Bombay rats carried out at the Haffkine Institute are given in Table VI.

Table VI

Species	Percentage of mortality					
	Bombay					Calcutta
	1952	1953	1954	1955	1956	1953-54
R. rattus ..	12·6	13·7	12·1	16·0	7·5	87·0
R. norvegicus ..	—	5·8	3·3	2·6	2·2	94·7
B. bengalensis Kok ..	77·6	82·5	76·7	70·0	75·2	97·6

It will be seen that between 1952 and 1956 there is a progressive increase of resistance in *R. rattus* and *R. norvegicus* but these two species are being replaced by the more susceptible species, *B. bengalensis* Kok, which now constitutes nearly 50 per cent of the rat population in the city. In Calcutta only *R. rattus* is found partially resistant but the population has recently been reduced to half of what was in 1906 or 1948. Recently Haffkine Institute was asked to regularly examine the rodents collected from different states in India for resistance and except those sent from the Madras city others have been found fairly resistant. This observation is in favour of the view that development of herd resistance of rats in different areas in the country has been partly responsible for the disappearance of plague, at least temporarily.

Vector Fleas

The two subfamilies of fleas called *Pulicidas* and *Coratephyllidae* are of great importance so far as plague is concerned. As with the rodents, the various species of fleas parasitizing wild, peridomestic and commensal rodents in different parts of the world, harbouring or suspected of harbouring plague infection, have been listed. Also the bionomics and ecology of this common species have been studied. A large volume of information is already

available regarding their growth and development, host selectivity, breeding habits, hibernation, biting habits, relative distribution in different parts of the country (local distribution), infectivity, mechanism of transmission of plague infection (blocked fleas, faeces, per os, mechanical, etc.), climatic and environmental influences, vector incidence and vector efficiency, longevity, role of infected and uninfected fleas, transportation of fleas in the spread of plague, role of wild rodent fleas, interchange of fleas between wild and domestic rodents, and so on. Besides, the role of other insects including the *Pulex irritans*—the human flea—has also been studied.

In India, the species of fleas found are : *X. cheopis*, *X. brasiliensis* and *X. astia* and *Ctenocephalus felis* and *canis*. The first two species are efficient vectors of plague infection while *X. astia* is a poor vector. In fact, one of the reasons of absence of plague in the Madras city given by King and Pandit (1931) was the absence of *X. cheopis*, the only species found being *X. astia*. The types of fleas and their distribution in the five cities of India at different times are given in Table VII.

TABLE VII

Types of fleas and their distribution in different cities of India

Places	Observer	Per cent of distribution				
		X. cheopis	*X. astia*	*X. brasiliensis*	*Ctenocephalus*	*P. irritans*
Calcutta	Strickland and Roy (1930) ..	40·0	60·0	—	—	—
	Rao (1936) ..	40·4	59·6	—	—	—
	Seal and Bhattacharji (1948–50)	34·4	65·6	—	4 fleas	—
Bombay	Cragg (1920) ..	49·5	49·8	0·7	—	—
	Cragg (1922-23)	53·1	45·8	1·0	—	—
	Webster and Chitre (1930) ..	69·6	27·9	3·3	—	—
	Deoras and Tonpi (1956) ..	76·3	23·7	—	—	—
Madras	King and Pandit (1931) ..	5·6	94·3	1 flea	1 flea	1 flea

Table VII shows that the distribution of *X. cheopis* and *X. astia* varies in different places, Calcutta having predominance of *X. astia* (65·6 per cent), Bombay of *X. cheopis* (76·3 per cent) and Madras almost wholly *X. astia* (94·3 per cent). A changing phase is also noticeable particularly in Bombay. In 1920, the city had almost equal distribution between the two fleas, a change was noticed in 1930 and it is still being maintained, *X. cheopis* constituting

at least three-fourths of the flea population. Besides, *X. brasiliensis* which was prevalent in low percentage has now practically disappeared. It may be mentioned here that the Madras city has always been free from plague. Along with the change in rat population in the city, the resistant *R. rattus* is being gradually replaced by the more susceptible *B. bengalensis*, and with this high rate of *X. cheopis* population the city of Bombay is running a potential risk of plague recrudescence.

Recent studies on the bionomics of fleas (Seal and Bhattacharji 1960*a*) show that while the reproduction goes on all the year round *X. cheopis*, the vector species, has two principal waves of growth—one in the winter months immediately preceding or covering the epidemic wave of plague, also indicated by higher flea index, and the other a smaller one during the rainy season, whereas *X. astia* has only one main wave of growth in the rainy season, extending to autumn. Deoras and Tonpi (1956) had similar experience in Bombay. While the peak of *X. cheopis* incidence was between March and May that of the *astia* was between September and December, the corresponding incidence of the other species being lowest during those periods. A concomitant change took place in the sex ratio also. For instance, during the peak incidence of *X. cheopis* the females increased greatly in population than the males and the proportion of *X. astia* was just the reverse at that time. In regard to the biting habits, *X. cheopis* is normally a poor feeder on man but the propensity definitely increases during the winter and spring both for human and rat blood. Furthermore, a comparative field study of the plague-free and plague-endemic wards in a city shows that the relative distribution of the species of rodents and fleas definitely influences the epizootic conditions (Seal 1954*b*).

Resistance in flea population

During recent years a number of communications reporting on the resistance of certain insects to DDT have been reviewed by Busvine (1957). Kilpatrick and Fay (1952) reported resistance in certain fleas to DDT but not to 5 per cent chlordane, while Wilson *et al.* (1957) noted that the fleas had become resistant to chlordane also. In India, no actual resistance of flea population was reported till 1958. But a few human cases having occurred in certain villages in Mysore, the local flea population was examined at the Malaria Institute of India and was found to have developed fair amount of resistance. In this regard a systematic study was undertaken by Patel *et al.* (1960) in the Bombay State. They observed high degree of DDT resistance in the Poona strain of *X. cheopis* being 19 to 5,000 times more resistant than the Satara strain. The interesting fact is that these strains became partially resistant to BHC (8 to 16 times) and dieldrin (2 to 3 times) although these

insecticides were not used in Poona. This resistance has apparently developed due to the spraying of wettable DDT (0·5–1 gm. per m^2) 2 to 3 times in the malaria season of the year. The concentration of DDT having deteriorated soon led to the development of resistance among the survivors. Nevertheless, it appears that the fleas in other areas may have been similarly affected and it is now essential to test them for resistance, so as to adopt suitable steps before the situation deteriorates further.

Mechanism of persistence of infection

Four years' continuous natural transmission experiment through *X. cheopis* carried out by the author (Seal 1957, 1960*c*) with both susceptible and partially immunized *R. rattus* and *B. bengalensis* on the lines of experimental epidemiology after Greenwood *et al.* (1936) has led to the conclusion that following an epizootis the plague infection may be maintained for prolonged periods in an inapparent or sub-clinical form in the commensal or peridomestic rodents and that depending upon the environmental and other ancillary conditions these inapparent foci (usually in the spleen) may lead to a sort of relapse with bacteriaemia followed by clinical plague and death or recovery with rise of immunity. Fleas play only the role of a vector in the matter of transmission and a temporary reservoir at best. It was also noted that in the perpetuation of infection, organisms of low virulence played more important role than the virulent organisms. Thus this experiment has provided some evidence to show that one of the mechanisms by which the inter-epidemic period is bridged over is the carrying over of infection, by the partially-resistant commensal and peridomestic rodents in the urban areas.

The Plague Bacillus

Since the discovery of plague bacillus by Kitasato (1894) and Yersin (1894) the problem related to its cultural methods, morphology, virulence, toxicity, antigenic structure, biochemical behaviours, serology and its differentiation with similar organisms particularly *P. pseudotuberculosis* have been studied and many obscure points clarified. Some studies have also been made of its character during epidemic and inter-epidemic periods. But the essential difference between the organisms of bubonic and pneumonic plague has not yet been elucidated.

(i) *Morphology and growth characteristics*

The so-called normal and involution forms as described by the earlier workers have now been found to be caused purely by unsuitable extrinsic conditions, as in older infection, primary buboes, decomposed carcasses or

unsuitable culture medium. Cultivated in a properly nutritive medium such as enriched casein hydrolysate broth or agar (Seal and Mukherji 1950; Seal 1950), sheep or rabbit blood agar (Sokhey 1939) or beef heart agar (Meyer 1948), etc., the growth is uniform, smooth and profuse. There is hardly any lag phase or auto-agglutination. On the above solid media the freshly-isolated virulent organisms show smooth, convex, viscous and often dew-drop-like colonies with or without a fringe. Only a rough and avirulent form shows a little different colony and morphological character. Another important point that emerged out of the above studies is that the plague-organism undergoes quick dissociation on repeated subculture particularly in an unsuitable medium and gradually loses its protective antigenic properties. The classical stalactite growth is therefore an indication of roughness and degeneration of the organism.

(ii) *Nutrition of plague bacillus*

The nutrition of plague bacillus is the essential prerequisite for all plague studies because on this depend its cultural, morphological, biochemical, antigenic, virulence and other differential characters. It does not grow in ordinary agar or broth medium unless seriously degenerated. Certain additional nutritive and accessory growth factors are necessary for its full development including its antigenicity (Rae 1939, 1940). This was the basis of the author's own work in some of his investigations (Seal and Mukherji 1950; Seal 1950, 1951*a*, *d*, *e*), because it is extremely important from the point of view of maintenance of the organism without losing its virulence and antigenicity for the preparation of vaccine. Jackson and Burrows (1956) have recently showed the virulence enhancing effect of iron on non-pigment (relatively avirulent) mutants of virulent plague strains. The enriched casein hydrolysate medium of the author (Seal and Mukherji 1950) not only contains iron but also traces of Ca, P, Mg and small amount of liver extract. One of the criteria of nutritive medium is the virtual absence of the so-called lag phase as noted in case of the enriched casein hydrolysate broth.

For maintenance of the organism freeze-drying in 5 per cent gum acacia has given excellent results in the author's hand (Seal and Habbu 1943). Alternatively, point culture on 5 per cent rabbit blood agar, slant or stab-culture in solid medium of the same composition kept sealed in cold room, maintains the full character of the organism for more than a year.

(iii) *Antigenic structure*

Prior to 1940 the plague workers were facing certain difficulties in regard to the physical, chemical, serological and immunological properties of plague

bacillus and the related organism like *P. pseudotuberculosis*. The particular difficulty was in regard to its serological behaviour and its differentiation with *P. pseudotuberculosis*, as the success of field endemiology and the detection of reservoirs during the inter-epidemic period largely depended on them. Investigation made by various workers from time to time (Sokhey and Maurice 1936; Schutze 1939; Bhatnagar 1940; Jawetz and Meyer 1944; Seal 1950, 1951*b*, *c*, *d*, *e*, 1952 and 1953; Devignat 1951; Girard 1953; Burrows and Bacon 1956) have greatly clarified the position.

The plague bacillus is now primarily divided into (*a*) virulent and (*b*) avirulent forms. The latter is again subdivided into (i) protective and (ii) non-protective strains, based on animal tests. The bacillus loses its virulence not only by cultivating it in artificial culture medium but also in the immune rats during the inter-epidemic period as observed by the author (Lal and Seal 1949; Seal 1958*c*). Serologically, the author (Seal 1951) was able to differentiate the virulent from the avirulent non-protective plague and pseudotuberculosis organisms but no differentiation could be made between the virulent and avirulent protective strains except by animal test and quartz ultraviolet spectrographic readings of the respective specific proteins. The other methods of differentiation between the plague and pseudotuberculosis organisms are: culturally, *P. pseudotuberculosis*, unlike virulent *P. pestis*, grows easily on agar slope, shows motility in stab culture, ferments glycerol and rhamnose, reduces malachite green and methylene blue, and so on (Seal 1952). Englesberg *et al.* (1954) concluded that virulence was determined by the quantitative relationship between envelope and toxin production.

Recently, however, Burrows and Bacon (1956) by a special technique have discovered two additional antigens called V and W which can differentiate virulent from the non-virulent plague strains being present in the former and absent in the latter. In a further extension of the study Burrows (1959) obtained two additional determinants of virulence, namely P (pigment positive) and Pu (purine producing). The V antigen is related to the property of the related organism by which it resists phagocytosis by mouse polymorphs. He has also studied both freshly-isolated and laboratory strains of *P. pseudotuberculosis* for the same antigens and found that the main difference between the two organisms was the absence of Fl in the pseudotuberculosis strains. The other three antigens are the same as *P. pestis* except that in some strains any one of them may be absent and, like *P. pestis*, the virulence can be enhanced with iron under similar conditions. Accordingly the serological antigenic structures of the plague and pseudotuberculosis organisms stand as follows :

Organism	Antigens	
P. pestis virulent	Fl+*, VW+, P+, Pu+,	common rough somatic antigen
P. pestis avirulent protective	Fl+, VW—, P+ or P—	Pu+, common rough somatic antigen
P. pestis avirulent non-protective	Fl—, VW—, P— or P+	Pu—, common rough somatic antigen
P. pseudotuberculosis virulent (fresh)	Fl—, VW+, P+, Pu+,	flagellar smooth and somatic smooth (type and group specified) common rough smooth
P. pseudotuberculosis virulent (laboratory)	Fl—, VW—, P+, Pu+,	

* Fl of Baker *et al.* is the same as A of Seal and relates to the envelope substance.

Devignat (1951, 1958), on the other hand, classified all the world strains into three noso-geographical varieties, based on their biochemical behaviour and on clinico-pathological effects (virulence). These are :

(i) *Var orientalis*, which does not ferment glycerine, transforms nitrates into nitrites and produces nitric acid in broth without nitrates and causes late septicaemia in mice; found in India, South China, Morocco, Madagascar, Indonesia, South Africa and U.S.A. This type corresponds to Berlin and Borzenkov's (1938) oceanic strains.

(ii) *Var mediavelis*, which ferments glycerine, does not transform the nitrates and does not produce nitric acid; seems to be to some extent pneumotropic; found in South-East Russia and Kurdistan.

(iii) *Var antiqua*, which ferments glycerine, transforms the nitrates but does not produce nitric acid; shows a tendency to provoke septicaemia; found in North-East Asia, North China and Belgian Congo.

Capsule and envelope

Controversy still exists in regard to the term envelope antigen and the capsule. Kurauchi and Homma (1938) called it 'capsular antigen', and Chertnik (1940) 'membrane antigen'. Previously Rowland (1914) postulated that capsule was present in certain circumstances only but Sokhey (1940)

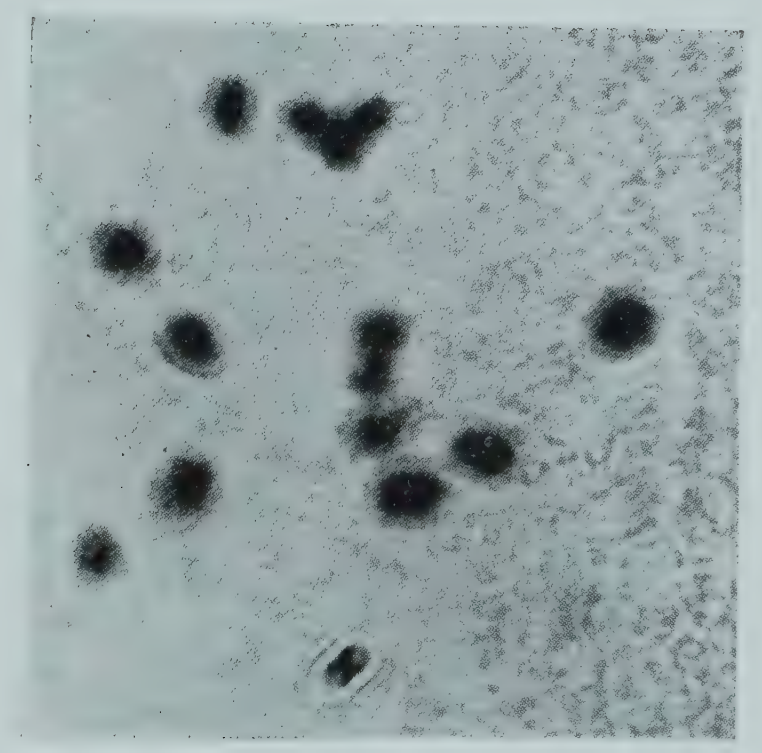
concluded that the plague bacillus possessed a capsule under all circumstances and the envelope seemed to be only an unstained capsule. In 1951, Amies called the envelope as nothing but partially well-developed bacterial capsule.

The author (Seal 1959*a*, *c*) carried out some intensive study on this problem and finally succeeded in defining both of them in the same specimen of virulent *P. pestis* by an ingenuous method of staining procedure (*see* photograph, Pl. XVIII). Secondly, while the protective avirulent plague strains like the Java and Madagascar (EV) strains also contain the envelope substance the non-protective avirulent strains are without it.

Toxin of the plague bacillus and enzyme studies

Rowland (1910) originally suggested that the plague bacillus had an endotoxin associated with the soluble protein. A few workers also suggested that it is a mixture of metabolic and disintegration products of the bacillus. Clinically also, the plague patients show various degrees of toxaemia. According to Girard (1941) and Girard and Sander (1947) plague endotoxin is similar to exotoxin, being thermolabile and convertible into toxoid, while Baker *et al.* (1952) claimed that Fraction II of specific soluble protein of plague bacillus (isolated between 0·4 and 0·57 saturation of ammonium sulphate) contained the toxic fraction but the identity of this substance was not clear. A very useful work has recently been done by Ajl *et al.* (1955, 1958). These workers either used casein hydrolysate mineral glucose medium (CHMG) and the Tjs strain or the autolysates of agar grown, acetone-killed dried bacilli of virulent strain 1951 P or attenuated EV 76. Partial purification was achieved by fractionation with 0·35 to 0·70 saturation of ammonium sulfate in the final purification by continuous flow paper electrophoresis or by electrochromatographic methods. The intravenous LD 50 in mice varied from 0·1 to 0·3 μg. The toxin could be denatured by physical or chemical agent with decrease or eventual disappearance of toxicity, but the formalin-treated toxin retained its ability to react with its specific antiserum while the enzyme-hydrolysis with trypsin, chymetrypsin and papain did not markedly affect the toxic or the serological activity of toxin in spite of a considerable liberation of free amino-acids. This finding will undoubtedly facilitate the production of specific antitoxin.

On the other hand, Jawetz and Meyer (1944) suggested that the toxicity may be associated with the enzyme make-up of the organism. Rechenmacher (1949) found greater catalase activity in the virulent organism than in the avirulent. A group of workers under Dr. Shrivastava (Sagar *et al.* 1956; Saxena *et al.* 1957; Srikantan *et al.* 1957 and 1958) also studied (1) deamination of amino-acids, (2) alkaline phosphatase activity, (3) oxidation metabolism, (4) transamination reaction, and (5) dehydrogenase in both virulent and



Photograph of stained *P. pestis* showing both capsule and envelope.

avirulent strains of *P. pestis*. In addition they have studied the action of certain antibiotics and sulfa-drugs on the oxidative metabolism and transaminase reactions. The optimum temperature for these studies was 37·5° C. The results, however, indicate that although the enzyme studies did not so far give any clue to the difference between virulence and avirulence these might yield other interesting results.

Chemical antigenic structure

The work of the earlier workers (Lustig and Galleoti 1900; Rowland 1910, 1914; Brooks 1912; Morison *et al.* 1924) sufficiently indicated that the plague bacillus was composed of at least two varieties of proteins—one was soluble in distilled water or saline and contained the immunizing and the toxic substance and the other was insoluble in water and salt solution without any specific immunizing properties. Following the early attempts of Shrivastava (1939), Seal (1943, 1950, 1951*d*, 1953, 1954*a*), Baker *et al.* (1947, 1952), Amies (1951) and Bhagavan *et al.* (1955) have greatly advanced our knowledge on the chemical antigenic structures of the organism, using different methods of isolation and purification.

The author (1951*e*) succeeded in isolating a polysaccharide yielding osagone resembling that of Arabinose (melting point 166–168° C.) from the supernatant of the Haffkine plague vaccine as well as from the specific soluble protein and, to a lesser extent, from the bacterial debris of both virulent and avirulent protective strains. It was found absent in non-protective avirulent plague and pseudotuberculosis organism. The author therefore concluded that the protective substance of plague bacillus was a polysaccharide-protein complex probably a nucleo-protein. On the other hand, Korobkova and her colleagues (1951) are reported to have isolated two different apparently impure polysaccharides in *P. pestis*, reacting slowly with both antiplague and anti-pseudotuberculosis sera.

Without going into the details of the various fractions isolated it may be stated that in practice there is a good amount of uniformity in the findings of the above workers on the basic antigenic structure of plague bacillus and its dissociants in spite of the apparent diversity. Probably the differences noted were due mainly to the materials and methods used. The PD's (protective dose) of the fractions A and B of Bhagavan *et al.* were 8 or 8·3 micrograms against a challenge dose of 160 mld's, that of Baker *et al.* 12 to 22 micrograms against 100 mld's and that of Seal 0·6 to 2·5 micrograms against 13 mld's. The author, however, recently retested the antigen A and Baker *et al.*'s antigen (combined IA and IB) against a challenge dose of 5,000 virulent organisms (*P. pestis* 195/P) in *R. rattus* and *B. bengalensis* caught in Calcutta. The results are given in Table VIII.

TABLE VIII

Results of immunization of rats with different protein fractions of P. pestis

Antigen	Immunizing dose	Survivals in immunized	
		R. rattus	*B. bengalensis*
Antigen A of Seal ..	0·1 mg.	19/20	18/20
Antigen I of Baker *et al.*	0·1 mg.	16/20	14/20
Antiplague vaccine in enriched CH broth 1,000 millia organisms/ml. ..	0·4 mg.	10/20	9/20

N.B.—Given in two doses at weekly intervals; challenge dose organisms (195/P.)

It appears that fractions A of Seal, IA and IB of Baker *et al.*, A of Bhagavan, etc., and the fraction I of Amies are all obtained from the envelope and/or capsular antigen of *P. pestis* as all of them have suggested. All these fractions are equally specific and highly protective against virulent plague infections. According to Baker *et al.* Seal's antigen may be a mixture of their IA and IB fractions, IA having the carbohydrate moiety as in Seal's antigen and IB and Amies' fraction being same as IA but without carbohydrate moiety.

In the words of Amies, not much importance need be given to the apparent discrepancies but what is needed is to evolve a standard technique for mass production of this specific antigen for human immunization.

Serodiagnostic procedures

The problem of serodiagnosis of plague infection in man or animal was fraught with difficulties for three main reasons, viz. (1) plague strains often formed unstable suspension in normal saline, (2) the antiplague serum raised against the whole organism acted against both plague and pseudotuberculosis organism, and (3) agglutination titre of the same organism differed at different temperatures of incubation. The author reinvestigated this problem in 1941-42 and evolved a technique by which all the difficulties were overcome. The agglutination is done with live suspension of organism grown on 5 per cent rabbit blood agar for 24–48 hours at 37° C. overnight. Two types of reactions occur: (1) floccular or woolly, and (2) granular, also described by Jawetz and Meyer (1944). The former is related to the envelope or protective antigen and gives low titre and the latter which is related to somatic antigen gives high titre agglutination. By using different antisera including the one against

the specific protein fraction A, absorbed or unabsorbed, the author (Seal 1951*b*) worked out the serological relationship between the different strains as given in Table IX.

TABLE IX

Serological relationship between P. pestis *and* P. pseudotuberculosis

Antisera produced against	*P. pestis* virulent and av. protective	Av. non-protective	*P. pseudo-tuberculosis*
Virulent *P. pestis* ..	+	+	+
Virulent *P. pestis* and absorbed with *P. pseudo-tuberculosis*	+	0	0
P. pestis boiled for ½ hour ..	0	+	+
P. pseudotuberculosis ..	0	+	+
Water-extractable protein ..	+	0	0
P. pseudotuberculosis and absorbed with *P. pestis* boiled	0	0	+

(*a*) *Serological test*

This serological test can also be used with fairly reliable result for the retrospective diagnosis of human cases of plague. The results of agglutination test in bacteriologically positive and suspected human cases of plague as carried out by the author in 1949 are given in Table X. For diagnosis of human plague cases Panja and Gupta (1948, 1949) used slide agglutination

TABLE X

Results of agglutination test in bacteriologically positive and suspected human cases of plague in 1949

Nature of cases	Number tested	Date of collection after onset	Date of examination after onset	Number positive	Per cent positive	Agglutination titre
Bact. ..	26	7–40 days	26–133 days	23 (3 doubtful)	88·4	1/10 to 1/200
Suspected cases (bact. negative or not done)	56	6–36 days	24–134 days	5	9·0	1/10 to 1/100
Non-plague cases	55	—	—	2 (both inoculated)	3·6	1/25 and 1/50

N.B.—The above results have been obtained in spite of sulphonamide and streptomycin treatment.

test with serum dilution of 1 : 3 or 1 : 4, usually on the seventh day after onset. But for earlier diagnosis bubo puncture fluid and sometimes blood culleni yield quicker result.

(*b*) *Precipitation*

The precipitation test could not be made popular for want of proper antigen. The isolation of the specific protein fractions by various workers described earlier opened the prospect of utilizing this technique as well for (1) serodiagnosis of plague infection in human beings and rats, (2) identification of plague strains, and (3) quantitative estimation of potency of antiplague serum. These methods have been actually utilized for plague studies (Seal 1951*c*, 1954*a*). For quicker diagnosis *ring precipitation* test may be done with patient's 1 : 2 to 1 : 10 sera against 1/1,000 dilution of Antigen A. Similarly, an unknown organism can be tested by growing the organism in CH broth and testing the filtrate against known antisera by the ring precipitation method. For qualitative precipitation the tube containing the mixture is incubated at 37° C. for 2 hours and then left in the refrigerator overnight. For quantitative test all the operations are carried out at 3–4° C. and incubated at that temperature for 24–48 hours (Seal 1954*a*).

(*c*) *Complement fixation test*

The earlier investigators Moses (1909), Damperoff (1910), Joltrain (1920), Simond (1898), Dickie (1926), Mitin (1938), and Wats *et al.* (1939) employed bacillar suspension or extracts as antigen but the results were not wholly satisfactory. The isolation of specific soluble protein fractions by the author and others greatly facilitated the performance of the complement fixation test using this antigen and high titre antiserum produced in rabbits against this antigen and it has been possible for the author (Seal 1953) and Chen *et al.* (1952) to (1) determine the evidence of plague infection in the tissue extracts of animals died of suspected plague, and (2) detect antibodies to fraction A or I in the sera of human convalescents and of immunized man and animal.

It may also be useful in the field diagnosis of wild rodent plague especially when the isolation of *P. pestis* or the interpretation of the pathological lesion at autopsy is rendered impossible by contamination or decomposition. Using this technique it has also been possible to differentiate between *P. pestis* and *P. pseudotuberculosis* and to detect antigenic deterioration of plague strains by cross-complement fixation test.

(*d*) *Flocculation*

Advantage has also been taken of the specific plague proteins in developing a flocculation technique for estimating the potency of antiplague

serum (Seal 1947). Based on the constant serum and variable antigen method between Antigen A and the various horse and rabbit antiplague sera the author succeeded in establishing a flocculation test giving results in 5–15 minutes at 45° C. This technique may be suitable for estimating the potency of antitoxin raised against Ajl *et al.*'s plague toxin.

Treatment of Plague

(*a*) *Serotherapy*

The only treatment before the advent of chemotherapy with sulfa-drugs worth mention was the use of antiplague serum and the iodine solution. In the earlier years Choksey (1900) reported 60 per cent success in non-septicaemic cases. Considerable difficulty was encountered in producing a potent and effective antiplague serum. Following Naidu and Mackie (1931), Sokhey greatly improved the production of antiplague horse serum between 1936 and 1939 at the Haffkine Institute, but by that time sulfa-drug was about to come. Sokhey also developed a standard method of assay of this serum in white mice (Sokhey and Maurice 1935).

The most effective antiplague sera available recently are the ones produced against avirulent EV Madagascar strain and the Haffkine Institute antiplague serum. These have been used on a fairly large scale with better results. Girard (1941) and Le Gall (1943) reported about 60–65 per cent cure with EV antiplague serum and Sokhey and his collaborators, 76·5 per cent overall cure in 157 cases but taking only 71 bacteriaemic cases the rate of cure was only 49·3 per cent while only one death was registered in the remaining 86 cases. The pooled experience of the recent uses of antiplague serum as summarized by Meyer *et al.* (1952) is given in Table XI.

Table XI

Summary of the reported results of treatment with antiplague serum

Area or place	Serum-treated cases		Serum-untreated cases	
	Number	Fatality rate	Number	Fatality rate
Eastern Hemisphere (India, Indonesia, Japan, Middle East, Africa and Madagascar)	3,840	45·13	1,726	83·5
Western Hemisphere (North America, Argentina, Brazil and Peru)	19,540	32·75	213	74·07
Haffkine Institute (1911–43) ..	320 (71 bacteriaemic cases)	25·0 (50·7)	135 —	63·7 —

It is now considered that serotherapy with antitoxin might be more useful than the antiplague serum and that serum raised in rabbits is more potent than that raised in horses (Korobkova 1937; Meyer 1947; Seal 1954*b*). Recently Semerova *et al.* (1957) and particularly Khundanov *et al.* (1958) found the gamma-globulin fraction of antiplague serum to be more efficacious than the original antiplague serum as well as its beta-globulin and pooled globulin fractions in guineapigs.

(*b*) *Chemotherapy*

The earliest compound tried was prontensil in 1938 by Carman (1938) and Vine (1928) and sulfanilamide by Van Hoof. Schutze (1932, 1939) found sulfapyridine better than sulfanilamide. Soon sulfathiazole was discovered followed successively by sulfadiazine, sulfamerazine and sulfamezathine. All were used for the treatment of plague in their turn. The combined experience of all workers including that of Sokhey, Wagle and others is given in Table XII. Of all the sulfa-drugs sulfamerazine seems to be superior to even sulfadiazine which has been more extensively used.

(*c*) *Antibiotic treatment*

Meyer and Quan first tried streptomycin in plague-infected animals in 1944. It seems Videalla tried it first in human cases in 1946. Comparative field trial with streptomycin was actually started in India in 1948. The rate of cure varied between 80 and 100 per cent. The total cases treated between 1945 and 1953 were 786 with only 33 deaths, a cure ratio of 95·8 per cent. These cases included septicaemic and meningeal plague also. The other antibiotics tried were aureomycin, chloramphenical, terramycin, neomycin and viomycin, etc., with good results, neomycin giving the best promise. Penicillin has also been used to check secondary infections. In the treatment of bubonic cases, however, sulfa-drugs may be enough. Very recently Semerova *et al.* (1957) used bacteriomycin with nearly as good result as streptomycin in plague-infected guineapigs.

Treatment of Primary Pneumonic Cases

Streptomycin had more striking results than sulfadiazine. Sometimes streptomycin with sulfa-drugs and antiserum have been tried with equal success in serious cases.

From the above records it can now be safely stated that effective treatment has been found in all types of plague cases including the primary pneumonic type. Thus the dread of plague has now been nearly completely removed.

TABLE XII

The combined results of treatment with sulfa-drugs (Indian and American data)

Drugs	Sokhey and Wagle (1941–49)		Simeons and Chittre (1946–47)		Datta Gupta (1948)		Combined results (India)		Meyer *et al.* (1952)	
	Cases	Per cent survived	Cases	Per cent survived	Cases	Per cent survived	Cases	Per cent survived	Cases	Per cent survived
Sulfanilamide ..	—	—	—	—	—	—	—	—	125	68·0
Sulfapyridine ..	122	73·0	—	—	—	—	122	73·0	725	45·4
Sulfathiazole ..	345	77·1	142	81·7	—	—	437	78·4	769	79·5
Sulfadiazine ..	168	91·5	704	82·0	41	90·1	1,097	83·8	1,061	91·4
Sulfamiazine ..	149	92·1	700	86·0	—	—	849	87·4	72	91·7
Sulfamezathine ..	—	—	—	—	37	89·2	37	89·2	—	—
Antiplague serum ..	157	76·5	—	—	—	—	157	76·5	—	—
Iodine soluble ..	149	46·3	—	—	—	—	149	46·3		
Combined sulfathiazole and antiserum ..	60	80·0	—	—	—	—	60	80·0	—	—

vaccine has also been used in Argentina, Belgian Congo, Brazil, French West Africa, Tunisia and Union of South Africa (Pollitzer 1954). But Sokhey and his coworkers in India did not agree to try such vaccines due to certain amount of risk involved such as the possibility of changing into virulent form. Besides, greater control and supervision for its preparation and better storing facilities are necessary. Also, without freeze-drying, it is not possible to keep the live vaccine for its use in distant parts of this tropical country.

Specific soluble protein for human immunization

The author (1943, 1951*b*, 1953) produced experimental evidence to show that the immunizing substance in the Haffkine plague vaccine is a specific soluble protein and the potency of the vaccine depends entirely on this specific substance. According to Meyer 2-3 mg. of this specific antigen is capable of producing a high protective immunity in man. The possibility of its use in human immunization against plague infection needs to be explored. Even the bacteria-free filtrate of casein hydrolysate broth vaccine may be dried and the crude protein may be used in quantities required for producing sufficient immunity. The present status of therapy of plague including the prophylactic vaccines has been thoroughly reviewed by the author (Seal 1960*c*).

Control Measures

Control measures can be divided into four main sections, viz. (1) control of plague in human beings, (2) control of rodents, (3) control of vectors, and (4) control of spread of plague at distance.

As already indicated considerable improvement has already been effected in the prevention and control of plague in human beings due to the discovery of effective drugs, prophylactic vaccines, therapeutic antisera and disinsectization methods. Rat-proofing of houses and godowns, control of movement of grains and of patients and contacts are adopted to stop spread of plague. The most important discovery which has directly influenced the control of plague is that of insecticide like DDT, BHC and DIELDRIN, etc. In India, the vector control has been effected not only through the dusting of burrows and the affected houses with 10 per cent DDT powder but the extensive antimalarial operation with wettable DDT suspension and emulsion seems to have given the collateral benefit of killing the fleas in the houses. Among the other insecticides chlordane, organic phosphates, pipernyl compounds and fumigants like HCN, cyanogas, etc., are also used. A study on the comparative pulicidal values of cyanogas, DDT and BHC by Wagle and Seal (1953) has shown that DDT is more efficient and economical insecticide among those so far as the common Indian vector fleas are concerned. Dieldrin

has also been found to be equally effective. The flea indices in different parts of India are showing a definite tendency towards decrease, the *cheopis* index hardly rising above 0·5 and this factor combined with that of herd resistance in the commensal rat mentioned earlier has been partly responsible for the gradual reduction of plague in India.

Resistance in flea population

Since several instances of development of resistance in flea population have recently been detected in India (in Mysore and Bombay States), the proposition should be met by an immediate change and intensive use of insecticide and a close vigilance kept in other areas so that the necessary steps may be taken on the slightest suspicion of resistance being developed in the flea population. Application of DDT in smaller doses than necessary for their control may ultimately prove to be dangerous and uneconomical. Although the present situation could not be avoided as antimalaria campaign had to run its own course, if antiflea measures are to be undertaken it should be done on a thorough basis with proper dose of the insecticide and should be spread over all the year round instead of depending upon the two or three rounds of DDT as a part of antimalaria operation. According to the recent work of the author and of Baltazard (1960) the reservoir of infection being the rodents, wild as well as commensals, the essential step needed for the eradication of plague is the systematic destruction of these rodents as has been done in U.S.S.R. (Fenyuk 1960). But so long as this is not possible due to financial reasons the main target of attack should remain with the vector fleas and wherever possible destruction of rats.

For the control of rodents, trapping, baiting, poisoning and various fumigants are in use. Among the fumigants, HCN, CO_2, CS_2, chlorpicrin and $Ca(CN)_2.SO_2$ have been successfully used. For poison-baiting a variable amount of success has been attained by using $BaCO_3$, arsenic preparations, phosphorus, red squill, zinc phosphate, antu, sodium fluoracetate and some anticoagulants. Virus and fungus infections have also been suggested. In fact, Russia seems to have launched a total eradication campaign against rodents as a whole and particularly against the wild rodents. According to the observations made by the author in Calcutta (Bhattacharji and Seal 1954) the normal range of movement of rats being 200 yards intensive antirat and anti-flea measures around 200 yards of the actual ratfall may be sufficient to control the spread provided the actions are promptly taken. As a long-term measure, however, slums, huts, and godowns which harbour rats should be replaced by pucca buildings or structures which would prevent rat harbourage. The success of control measures will depend upon the extent of improvement brought about in this respect.

Concluding Remarks

With the natural decline of plague either through the development of herd resistance in the commensal rats or by active antiplague measures it seems that the infection tried to recede to its original host reservoirs, namely the wild rodents, and it may be that the same phenomenon will follow in India, too, as recently noted by Baltazard *et al.* (1958). The author has already discussed about the epidemic behaviour of plague from the historical standpoint. It may now be stated that, although plague has often shown decline even when left alone, it flared up again in due course. But before a general recrudescence occurs a change or mutation takes place in the organism facing extinction as a natural process for survival. Thus the three varieties of plague organisms described by Devignat may be related to such changes prior to the three successive pandemics, and one never knows when the history will repeat itself. There is nothing therefore to be complacent about the disease, in spite of the great decline in the incidence at present. Rather this is the time to make certain fundamental studies on the evolution of plague in the fields and to prepare our weapons for better control of the situation which may yet arise under the secular behaviour of this fell disease. At the same time there seems to be no reason to fear as widespread outbursts are not likely to occur in the face of the knowledge and experiences gained about its treatment, prevention and control during the last half of a century. Nevertheless, from both national and international points of view priority should be given to the detection and elimination of foci of infection among the rodents, either commensal or wild. But so long as such a procedure does not become feasible, both antiflea and antirat measures should be continued in the places where plague either in rodents or in man has been recently reported and strict vigilance should be kept for any such incidence from any quarter, however small it might be. The vigilance should also include regular examination of rodents from different areas for possible infection and resistance against it and of flea population for resistance against the currently used insecticides, in addition to the strict observance of quarantine regulations.

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Appendix I

The chronological order of the development of knowledge about plague

1894 (14 June)	Discovery of *Pasteurella pestis* by Yersin and Kitasato from the blood, bubo and spleen of human patients.
1895	Antiplague serum by Yersin, Calmette and Borrel.
1896	Antiplague vaccine by Haffkine at Bombay.
1897 1898	Ogata (Formosa) Simond (India) } Fleas as vectors of transmission from rat to rat and man.
1902-3	Gauthier and Raybaud proved transmission by fleas experimentally.
1904	Dürk of Austrian Plague Commission worked out the pathological anatomy of plague.
1905	Glen Liston (Bombay) and the Indian Plague Research Commission confirmed rat-flea-rat transmission.
1910	Rowland postulated endotoxin of plague bacillus.
1914	Bacot and Martin (Indian Plague Research Commission) described the role of blocked flea in the transmission of plague infection.
1928	Jorge (Africa) described plague in wild rodents (Sylvatic plague).
1930	Naidu *et al.* utilized horses at Bombay for the production of antiserum, the only available remedy till that time.
1932	Schutze differentiated the envelope and somatic antigen.
1932–35	Sokhey at Bombay described mouse-protection test for the standardization of anti-plague vaccine and sera.
1935	Otten in Java introduced live attenuated plague vaccine followed by Girard and Robic in Madagascar and Pirie and Grasset in South Africa in 1938.
1939	Sokhey standardized the virulence test, culture medium and described optimum conditions of growth of plague bacillus.
1939	Schutze (Great Britain) and Sokhey (India) introduced treatment of plague with sulfa-drugs.

1940 Bhatnagar (Kasauli, India) classified the plague and pseudotuberculosis organisms by improvised agglutination test.

1940-41 Seal and Mukherji at Bombay utilized modified casein hydrolysate medium for the growth of plague strains and the study of chemical antigenic structure.
Seal also solved the problem of auto-agglutination of plague strains by using live organisms against the various types of antisera raised, and developed serological test for identification of virulent plague from non-protective plague and pseudotuberculosis strains.

1943 Seal isolated specific soluble protein fractia of the plague bacillus and also the fraction correlated with *P. pseudotuberculosis*.

1944 Jawetz and Meyer (U.S.A.) differentiated virulence from toxicity of plague bacillus and studied the mechanism of immunity in plague.

1946-47 Viswanathan (Bombay) used DDT as an effective insecticide in India.

1947 Baker *et al.* (U.S.A.) purified specific protein antigen of plague bacillus.

1947-48 Streptomycin was used very successfully in both bubonic and pneumonic plague in India and other places.

1949 Devignat (Belgian Congo) classified plague strains into *orientalis*, *antiqua* and *mediavelis* types on the basis of reaction on glycerine and production of nitrous acid.

1950 Sokhey *et al.* (Bombay) adopted casein hydrolysate medium for the preparation of antiplague vaccine.

1956 Burrows and Bacon described two additional antigens, V and W, in plague bacillus.

1952–57 Seal proved on the basis of experimental epidemiology that partially immune rodents act as reservoirs of infection during inter-epidemic period by harbouring plague organisms of reduced virulence in an inapparent form or in chronic foci in spleen and not by fleas under the Indian environmental conditions.

1959 Seal presented evidence of both capsule and envelope being present in virulent plague bacillus.

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